



## ORIGINAL ARTICLE

# Influence of Dosage and Compost Humification Degree in Nutrient Uptake by Blackberry Plants Grown in Greenhouse

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### ABSTRACT

The effect of sugar cane compost additions was evaluated in the nutrient uptake by blackberry (*Rubus spp.*) cultivated in greenhouse, in a Chromic Luvisol soil from the municipality of Ziracuaretiro, Michoacán, Mexico. Five compost dosage with five composting periods were mixed with the soil. They were applied 100-50-100 mg of N, P<sub>2</sub>O<sub>5</sub> y K<sub>2</sub>O respectively, by kg of soil-compost mixture. The treatments were carried out using a completely random distribution with four repetitions. The composts were characterized by chemical and physicochemical methods, the functional groups and the humic and fulvic acids were analyzed and the E<sub>4</sub>/E<sub>6</sub> and C/N ratios were estimated. The plants were harvested after 90 day of growth period and then, the macronutrients (N, P and K) and micronutrients (Cu, Zn, Mn and Fe) of the plant were analyzed. The higher compost dosage and humification period, cause higher macronutrients uptake, but when increases the compost dosage, decreases the micronutrients uptake

**Keywords:** Compost, blackberry, macronutrients, micronutrient retention.

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### INTRODUCTION

The soil nutrient dynamics related to plant growth is regulated by physical, chemical, physicochemical, microbiological and environmental factors; among the physicochemical factors is the rhizosphere pH, which regulates the micronutrient availability such as Fe, Cu, Zn and Mn [1]. Organic matter is another factor that influences the essential elements availability, its application improves the N availability because of the nitrifying bacteria action (*Nitrosomonas* and *Nitrobacter*) and that of others aerobic microorganisms which accelerate its degradation; in addition, the environmental changes which may affect plant growth with chemical conditions change [2]. Furthermore organic acids formation and other complex compounds immobilize some nutrients, since it has been found that when the C/N ratio is high, there is NO<sub>3</sub><sup>-</sup> retention and if waste does not contain sufficient organic N, the degrading microorganisms get it from the soil solution, reducing its plants availability that affect the crop nutrition [3].

During plants and waste degradation process a stabilized organic material is generated through a microbiological transformation process [4], which, by degrading hydrocarbon molecules, result in the formation of supra molecular humified component consisting of small humic molecules associations structured by hydrophilic and hydrophobic functional groups, which agglutinate soil clay to form micro aggregates [5] and change their structure, improve aeration, water retention capacity and crop production [6].

Due to its humic substances content, the organic amendments such as compost application, slowly release some essential nutrients to plants, such as N, P and S; in addition increase the P mobility [7, 8].

The humic substances application to crops such as grapes and cucumber has influenced on the increase of the macronutrients (N, P and K) and some micronutrients (Zn and Mn) contents compared to the controls [9]. On the other hand organic amendments additions improves the P availability for plant growth in acidic soils, because the competition between phosphates and organic acids released during its decomposition for adsorption sites on the soil colloids surface or dissolving some P rich secondary minerals [10]. In addition, the P consumers microorganisms are an available P source to plants during the humification process of organic amendments [11].

The oxygenated functional groups of humic substances (COOH, OCH<sub>3</sub>, C = O, OH phenolic) are electron donors and confer them a great ability to form metal complexes whose stability is given by the pH and soil organic matter chemical characteristics. Heavy metal binding to other soil components depends essentially on the pH and the presence of organic complexes, as has been tested with the fulvic and humic acids complexes formation with divalent and trivalent cations. Humic substances contained in the organic amendment such as the compost additions can reduce metal availability, and their effects on the Zn, Cu and Mn solubility depend on its humification degree and soil pH, salt content, CEC and redox changes in soil conditions because of the stable metal chelates [12].

The aim of this research was to evaluate the effect of increasing the dosage of sugar cane rum composts with different humification degrees on blackberry (*Rubus* spp., Brazos variety), over nutrient uptake in greenhouse conditions.

## MATERIALS AND METHODS

The present research was carried out with a Chromic Luvisol soil from municipality of Ziracuaretiro, Michoacán, México [13], on the premises of CIIDIR-IPN MICHOCÁN in Jiquilpan City, northwest of the Michoacán State, Mexico. Bounded by the parallels 19 ° 52'54 "and 20 ° 03'02" north latitude and 102 ° 39'33 "and 102 ° 56'16" west longitude and at an altitude of 1550 m. The climate is semi-warm subhumid with summer rains, with an annual rainfall of 326 mm and the maximum environment temperature from May to July is 27 - 30°C and the minimum from December to February is 9 -12°C on average [14]. The soil has a high organic matter content, therefore, a high content of N and low C/N ratio, however, the P, K, Ca and Mg levels are low [15] (Table I).

Table 1. Soil Chemical Analysis

pH <sub>H2O</sub>	OM %	C %	N %	C/N	P ppm	K	Ca	Mg
						Cmol <sub>c</sub> /kg		
6.05	5.80	3.37	0.29	11.63	4.00	5.29	6.00	2.25

OM-Organic Matter; ppm- parts per million; me-miliequivalent

The materials studied were organic solid wastes from the sugar mill of Taretan, Michoacan State, Mexico, which were subjected to a composting process with different humification degrees (0, 8, 12, 24, and 36 months respectively), these materials were characterized chemically (Table 2). The indicator crop of the organic matter influence on nutrient uptake was blackberry (*Rubus* spp., Brazos variety), which is characterized by a semi-erect plant, it grows well in regions with warm, dry summers under irrigation, requires deep and fertile soils with high organic matter content, pH between 5.5 and 6.5 and good drainage [16].

Table 2. Compost Physicochemical Analysis

Parameter	Compost humification time (months)				
	0	8	12	24	36
pH <sub>H2O</sub>	5.2	7.6	8.4	6.4	6.0
pH <sub>KCl</sub>	.1	7.3	8.1	6.3	5.9
ΔpH = pH <sub>KCl</sub> - pH <sub>H2O</sub>	-0.1	-0.3	-0.3	-0.1	-0.1
Humicacids (%)	4.17	8.80	1.24	12.95	8.92
Functional groups (me/100g)	20	500	664	481	438
-COOH (me/100g)	17	409	560	379	347
E <sub>4</sub> /E <sub>6</sub>	4.71	3.87	2.83	2.15	1.68
C (%)	56.7	52.3	48.2	35.4	32.4
N (%)	1.26	2.26	3.20	2.40	2.50
C/N	45.0	23.2	16.1	14.7	12.9
CEC (Cmol <sub>c</sub> /kg)	28.44	63.28	128.93	95.22	56.18

An experiment was conducted in a greenhouse to evaluate the response of blackberry (*Rubus* spp.) to additions of composts at different dosage (0, 1.875, 3.75, 7.5, and 15 g kg<sup>-1</sup> of soil) and with different humification periods (0, 8, 12, 24 and 36 humification month); a completely randomized design was used with four repetitions. To set the experiment five kg of air dried soil was placed in a container, previously

crumbled and sieved through a mesh of 2 mm, then air dried compost was added in the amounts mentioned.

The soil fertilization treatment used was 50-50-100 mg/kg as a source of nutrients, for N urea (46% N) for P, calcium triple superphosphate (46% P<sub>2</sub>O<sub>5</sub>) and for K, potassium chloride (60% K<sub>2</sub>O) were applied; once were available the three ingredients (soil, compost and fertilizer) these were mixed and watered to field capacity. Then two blackberry seedlings were transplanted per container, to allow the most vigorous one to grow. The plants were watered twice a week until harvest. Forty five days after transplantation was performed a second fertilization with the treatment 50-00-00 using urea (46% N) as N source in order to correct some N deficiency symptoms shown by plants grown in the sugar cane rum. Ninety days after transplantation they were harvested by cutting the plants two centimeters above the ground level. Blackberry plants were washed with water to remove soil particles, quickly rinsed with distilled water, dried in a forced circulation oven at 60° C to constant weight. The dry plants were ground in a porcelain roll mill and sieved with mesh No. 40.

#### Soil Analysis

The pH of a suspension soil-water (1:2.5 ratio) was measured, using a potentiometer with glass electrodes. N was determined by the Kjeldahl technique; the P by the Bray P1 technique; K, Ca and Mg using ammonium acetate pH 7.0 and micronutrients, with DTPA; the quantification of K, Ca and Mg was performed with spectrometry induction coupled plasma ICP-AES Varian<sup>TM</sup> Liberty II [17].

#### Tissue Analysis

The tissue chemical analysis was performed with a wet digestion using sulfuric acid + perchloric acid + nitric acid. The macronutrients (P, K, Ca and Mg) and micronutrients (Fe, Mn, Zn and Cu) were determined. The N was determined by semimicroKjeldahl technique; for P, K, Ca, Mg and micronutrients quantification, the induction spectrometry coupled plasma was used ICP-AES Varian<sup>TM</sup>, Liberty II [17]. The tissue chemical analysis was performed with a wet digestion using a mixture of sulfuric acid + perchloric acid + nitric acid. The macronutrients (P, K, Ca and Mg) and micronutrients (Fe, Mn, Zn and Cu) were determined. The N was determined by semimicroKjeldahl technique; the quantification of P, K, Ca, Mg and of micronutrients was performed by the induction spectrometry coupled plasma was used ICP-AES Varian<sup>TM</sup>, Liberty II [17].

#### Statistical Analysis

A variance analysis was done with the dry matter data and mean separation with Tukey test ( $P \leq 0.05$ ) such as described by Box *et al.* [18], also a regression analysis was performed to determine the relationship between compost dosage and dry matter production, to do this, the SAS package was used [19].

## RESULTS AND DISCUSSION

### Composts Physicochemical Analysis

The composts physicochemical analysis indicated that compost pH increases with increasing humification time, reaching its maximum value at 12 months of humification, and then decreases. Regarding the humic acids content it was observed that these follow the same trend that pH, only that its value was maximum at 24 humification months and then also decreases. The values of the functional groups, carboxylic groups, N and cation exchange capacity increased to reach its maximum value at 12 humification months and then decreased gradually towards the 36 humification months; the relationship between time of humification and C/N ratio was inverse and reached its minimum value at 36 months of compost humification (Table 2).

### Dry Matter Production

Dry matter production obtained was influenced by dosage and humification periods of applied compost, for example, the control (soil) had the lowest values (2.962 g plant<sup>-1</sup>) while the 3.75g·kg<sup>-1</sup> dose or higher, showed higher values, the sugar cane rum with a humification time of 0 h produced lower values, while the composts with 8 or more humification months had higher yields (Table II).

Variance Analysis indicated significant highly difference ( $P \leq 0.01$ ) when compared to the dry matter average yields produced with different compost doses and with different humification period. In general, when increasing both the compost dosage as the humification time, increased also the dry matter average yield per plant. For example, the plants grown in the compost with 36 months of humification at dosage of 15 g/kg, statistically they had the highest dry matter average yield (5.165 g/plant). On the contrary, plants grown in the sugar cane rum (without humification) had the lowest dry matter yields (Table 3).

The regression equations and determination coefficient ( $R^2$ ) were estimated for dry matter production of the crop against composts dosage and humification time (Table 4). The best fit to describe the increase in dry matter production in relation to the compost dosage for sugar cane rum was a third-order polynomial, with a tendency towards maximum production at a dosage of 3.75 g/kg soil. For compost

with 8 h of humification time, a second order polynomial was obtained, with a trend towards the maximum production at a dosage of 15 g/kg soil. For compost with 12 h of humification time, a third-order polynomial was obtained with a tendency toward the maximum production at a dosage of 3.75 g/kg soil. For the compost with 24 h of humification time, a second order polynomial was obtained with a tendency towards maximum production at a dosage of 7.50 g/kg soil and finally, for the compost with 36 h of humification time, a first order equation indicates that the production increased linearly at the dosage of 15 g/kg soil.

#### **Macronutrients Foliar Analysis**

In relation to the absorbed nutrients (N, P and K) by the blackberry plants grown with different compost dosage and with different humification times, it was observed that increasing both the dose of compost as its humification time, also increases the uptake of nutrients because with the increased of the compost maturity, its contents of N, P and K also increases ( Tables 5, 6 and 7) ; in the case of N, it is probably that the total N concentration increased during the humification process [20]. This N, P and K behavior is consistent with the observed by Xu and Saiers[21], they noticed that the uptake of these nutrients was increased according the increased N dosage from 0 to 500 kg/Ha with the celery crop; furthermore, the composts favored the conditions for the uptake of three macronutrients which were available in greater quantity, however the results found by Domeño et al. [3] differ from those obtained in this research, as they found that using organic substrates it showed N retention as a microbial activity results in g/plant.

In the case of phosphorus, the competition between phosphates and organic acids released during the humification of organic solid waste process, by adsorption sites on the soil colloids surface [10]. This is explained by considering inorganic P consumers microorganisms as a available P source to plants during the humification process of organic amendments. Organically amended soils may provide a greater percentage of residual P for crops [8]. Composts application resulted in an increase of P available which appears is related to the mineralization of the soluble organic and inorganic P, initially present in the compost. Citric and oxalic acids excreted by the roots or generated during the humification process of organic matter causes soil colloids phosphates desorption and organic complexing[22] through a ligands exchange, being free for the plants. The mechanism consists of the formation of a Fe-P-citrate whereby P is solubilized, in addition, the accumulated P in blackberry, in part, attributed to the increased supply of available P in composts, as well as the root growth promotion and P increased access to plants [11].

#### **Micronutrients Foliar Analysis**

Micronutrient uptake followed a different uptake pattern, in the case of Zn, Cu, Mn and Fe, when the compost dosage increased, decreased the amount of absorbed micronutrient by blackberry plants (Tables 8-11).

This micronutrients behavior in the soil-composts mix at different doses and humification degrees, in part, this is because some organic acids and ligands excreted from the roots, or generated during the humification process of soil organic matter, penetrate the humic substances superstructure, weaken the coercive forces of the hydrophobic groups and cause its disintegration to form humic molecules with smaller molecular size, enhancing the functional groups which, by cleavage of their H<sup>+</sup> increases the negative charges [23], thereby enhancing its ability to adsorb the micronutrients through metal complex formation without chelating character (they form insoluble complexes with Zn and Cu) [24], thereby decreasing mobility and availability to plants [6]. Furthermore, the effect of organic matter additions on the distribution of Zn forms depends on the pH, since at pH 5.8, decreases interchangeable Zn, and can be due to adsorption or organic complexation that immobilizes micronutrients [25], also humic acids can form Zn insoluble complexes unavailable to plants [26]. The pH effect can be modified by organic ligands which can decrease Zn uptake by the plant as the soil pH increases, such as in the present case, since the pH varied between 6.2 and 6.7; increases soil pH above 6.0 induce the hydrolysis of hydrated Cu, resulting in a strong Cu<sup>2+</sup> adsorption on clay minerals and organic matter that is the main constituent of soil which adsorb the Cu and complexing it quickly [24], reducing their availability; pH increases as decreases organic colloids size of high molecular weight and increases the surface on which the Cu, Mn and Fe can be adsorbed [24]. Mortvedt et al., [27] summarized studies of several authors about Cu in the soil and plant, indicate that it is retained by humic and fulvic acids through Electrovalent and covalent links, in CuOH<sup>+</sup> form in the carboxylic groups, and in the Cu<sup>2+</sup> form in the phenolic groups; in the last case the Cu is tightly bound, not available to the plants. The Cu solubility in the soil is decreased by complexation with clay-humus particles and/or insoluble humic complex formation. The mineralization of crop residues by soil microorganisms, release Cu, but some natural substances produced during the decomposition of organic matter trap it in ways not available. The Mn<sup>2+</sup> is retained by humic substances ligands and organic acids, phenolics, amino acids, hydroxamates, siderophores produced by organisms in the soil solution. Hydrated Mn complexes with carboxylic groups of organic matter may reduce their availability in soil [24]. Citric, malic, oxalic acids and phenol which form Fe soluble complexes are released when the organic

material decomposes, these complexes intensify the Fe mobility and bioavailability, but in the complexes of Fe and organic matter, its bioavailability is affected more by pH than by the organic content. Humic and fulvic ligands form the most stable complexes with Fe compared with other transition metals, and the effectiveness of these complexes is increased with pH increase due to increased ionization of the dispersion of surface ligands.

Table 3. Blackberry dry matter yield (g/plant) developed in composts with different dosage and different humification time in greenhouse

Humification time (months)	Compost dosage (g/kg)				
	0.000	1.875	3.750	7.500	15.000
0	2.962bA	3.150aB	3.465aC	2.677cD	2.455dD
8	2.732cA	3.300cB	3.860bC	4.220aB	4.537aB
12	2.927dA	3.292bA	4.847aA	3.742cC	3.785cC
24	2.915cA	3.835cC	4.585aA	4.625aA	4.255bB
36	2.837cA	4.017bA	4.317bB	4.335bA	5.165aA

Capital letters indicates significance between composts and lower letters indicates significance between doses.

Table 4. Regression equations corresponding to the composts studied

Humification time (months)	Mathematical Model	R <sup>2</sup>
0	$Y = 2,9096 - 0.3382X - 0.0726X^2 + 0.0032X^3$	0.9217
8	$Y = 2,7863 + 0.2948X - 0.0119X^2$	0.9840
12	$Y = 2,9094 + 1.0844X - 0.1836X^2 + 0.0077X^3$	0.9971
24	$Y = 2,7068 + 0.4432X - 0.0227X^2$	0.7710
36	$Y = 3,4374 + 0.1239X$	0.7628

Table 5. Nitrogen uptake (g/plant) by blackberry plants developed with different compost dosage and with humification times different

Humification time (months)	Compost dosage (g/kg)				
	0	1.875	3.75	7.5	15
0	1.39ABa	1.72Ab	1.42ABc	1.50ABb	1.14Bc
8	1.26Ca	2.03Bb	1.77Bb	1.87Bb	2.80Ab
12	1.35Ca	2.00Bb	2.57Aa	1.74Bb	2.18Bb
24	1.31Ca	1.27Cc	1.79Bb	1.93Bb	3.66Aa
36	1.33Ca	2.51Ba	2.35Ba	2.70ABa	3.63Aa

Capital letters indicates significance between composts dosage, while the lower case letters between compost with humification times different.

Table 6. Phosphorus uptake (g/plant) by blackberry plants developed with different compost dosage and with humification times different

Humification time (months)	Compost dosage (g/kg <sup>-1</sup> )				
	0	1.875	3.75	7.5	15
0	0.3433Ba	0.4038Bc	0.4571ABb	0.3986Bb	0.4991Ab
8	0.3188Ca	0.4362Bbc	0.4913ABb	0.5531Aab	0.5690Aab
12	0.3285Ca	0.5578Ba	0.6137Aa	0.5556Bab	0.5240Bab
24	0.3282Ba	0.3875Bc	0.6939Aa	0.6116Aa	0.6627Aa
36	0.3026Da	0.5132Cab	0.6599ABa	0.6189ABa	0.7067Aa

Capital letters indicates significance between composts dosage, while the lower case letters between compost with humification times different.

Table 7. Potassium adsorption (g/plant) by blackberry plants developed with different compost dosage and with humification times different

Humification time (months)	Compost dosage (g/kg)				
	0	1.875	3.75	7.5	15
0	2.49Ba	2.99ABc	3.37Ac	2.53Bb	2.31Bb
8	2.32Ca	3.28Bbc	3.53ABbc	3.87ABa	4.30Aa
12	2.49Ca	4.23Aa	4.44Aa	3.59Aa	3.88Ba
24	2.48Ba	3.04Bc	4.45Aa	4.32Aa	4.06Aa
36	0.97Ba	3.87Aab	4.33Aab	4.01Aa	4.83Aa

Capital letters indicates significance between composts dosage, while the lower case letters between compost with humification times different.

Table 8. Zinc uptake (g /plant) by blackberry plants developed with different compost dosage and with humification times different

Humification time (months)	Compost dosage (g Kg <sup>-1</sup> )				
	0	1.875	3.75	7.5	15
0	41.08A a	21.15 B a	17.25 C a	16.69 D a	9.43 E a
8	37.10A ab	10.20 B b	12.91 B bc	12.41 B ab	11.15 B a
12	38.14A ab	9.28 C b	14.74 B b	11.31 BC ab	9.56 C a
24	40.52 A a	6.44 C c	10.30 B cd	11.70 B ab	8.84 BC a
36	34.31 A b	9.10 B b	10.08 B d	10.16 B b	11.64 B a

Capital letters indicates significance between composts dosage, while the lower case letters between compost with humification times different.

Table 9. Copper uptake (g/plant) by blackberry plants developed with different compost dosage and with humification times different

Humification time (months)	Compost dosage (g/kg)				
	0	1.875	3.75	7.5	15
0	5.861Aa <sup>++</sup>	2.835Ba	2.287BCb	1.205Dc	1.866Cb
8	5.082Ab	2.376Cab	2.665Cab	1.604Dbc	3.539Ba
12	5.533Aab	2.146BCbc	2.666Bab	1.971BCa	1.628Cb
24	5.568Aab	1.644Dc	2.292Cb	2.220Ca	3.276Ba
36	5.079Ab	2.370BCb	2.806Ba	1.734Cabc	2.428BCb

Capital letters indicates significance between composts dosage, while the lower case letters between compost with humification times different.

Table 10. Manganese uptake (g/plant) by blackberry plants developed with different compost dosage and with humification times different

Humification time (months)	Compost dosage (g/kg)				
	0	1.875	3.75	7.5	15
0	20.868Aa	17.97Aab	16.473Ac	15.93Aa	15.553Aa
8	18.909Aa	17.952Aab	17.111Abc	17.243Aa	16.607Aa
12	21.166Aa	20.99Aa	21.184Aa	16.322Ba	17.132Ba
24	19.997ABa	18.281ABab	18.403Aab	17.414ABa	16.552Ba
36	20.868Aa	19.97Bb	19.473ABbc	18.93ABa	17.553ABa

Capital letters indicates significance between composts dosage, while the lower case letters between compost with humification times different.

Table 11. Iron uptake (g/plant) by blackberry plants developed with different compost dosage and with humification times different

Humification time (months)	Compost dosage (g/kg)				
	0	1.875	3.75	7.5	15
0	123Aa <sup>++</sup>	100.2ABa	87.9ABa	92.4Ba	95.3Ba
8	123.4Aa	78.1Cb	81.9BCabc	93.5BCa	95.4Ba
12	122.1Aa	70.1Bc	73.6Bbc	77.5Ba	82.3Ba
24	123.3Aa	85.1BCabc	70.5CDc	89.4Ba	56.8Db
36	121.7Aa	90.5Bab	84.4BCab	79.4BCa	74.7Cab

Capital letters indicates significance between composts dosage, while the lower case letters between compost with humification times different.

## CONCLUSIONS

The sugarcane rum affects the plant blackberry growth. By increasing the compost dosage and its humification degree in blackberry growth, increased also the macronutrients (N, P and K) uptake, but decreased the micronutrients (Zn, Cu, Mn and Fe) availability and uptake by the blackberry plants, due to the greater retention of these by the compost.

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