Article

Nitrogen availability and biomass yield of *Ricinus communis* L. fertilized with biosolids

Sandra Patricia Maciel-Torres[1]
Uriel Figueroa-Viramontes[2]
María del Rosario Jacobo-Salcedo[3§]
Ricardo Trejo-Calzada[4]
Aurelio Pedroza-Sandoval[4]
Rubén Carlos Encerrado-Alva[5]

[1]General Terán Experimental Field-INIFAP. Montemorelos-China highway km 31. General Terán, Nuevo León, Mexico. CP. 67400. (maciel.sandra@inifap.gob.mx). [2]North Central Regional Research Center-INIFAP. Blvd. José Santos Valdés num. 1200, Matamoros, Coahuila, Mexico. CP. 27440 (figueroa.uriel@inifap.gob.mx). [3]National Center for Disciplinary Research RASPA-INIFAP. Right Bank Sacramento Canal, Gómez Palacio, Durango, Mexico. CP. 35140. [4]Regional Unit of Arid Zones-Chapingo Autonomous University. Gomez Palacio road-Chihuahua km 40, Bermejillo, Durango, Mexico. CP. 35230. (apedroza@chapingo.uruza.edu.mx; rtrejo@chapingo.uruza.edu.mx). [5]Faculty of Biological Sciences-Juárez University of the State of Durango. Av. University s/n Fractionation Philadelphia, Gómez Palacio, Durango, Mexico. CP. 35010. (saiiro_dcshoes@hotmail.com).

[§]Corresponding author: jacobo.rosario@inifap.gob.mx.

Abstract

A criterion to estimate the application dose of biosolids in agricultural soils is the available nitrogen and the requirement of the crop. The objective of the study was to determine the nitrogen availability of two biosolids generated from different treatment systems and its effect on the oil yield from *Ricinus communis* L. seed. The treatments consisted of three sources of nitrogen (N) and three doses, in addition to a control without N. The sources of N were: ammonium sulfate (SA), biosolids from oxidation lagoons (BLO) and biosolids from activated sludge (BLA). The SA was considered as a commercial witness. The doses of N were 0, 3.79, 5.65 and 7.52 g pot^{-1}, which correspond to 67%, 100% and 133% of the N requirement of the crop (RNC), respectively. The greenhouse experiment was established under a randomized block experimental design with four replications, where the experimental unit was one plant per pot. Nitrogen availability was estimated using the fertilizer equivalency method (EF). The dry matter (DM) was statistically similar between the treatments, being different from the control ($p< 0.05$). The DM and N extraction showed a quadratic response as a function of the applied N doses. The availability of N was 40.4 and 34.8% for the BLA and BLO, respectively.

Keywords: nitrogen extraction, organic fertilization, plant nutrition.

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Introduction

In Mexico, in 2014 there was a record of 2 337 wastewater treatment plants (WWTP), which allowed a national coverage in municipal wastewater treatment of 52.7% (CONAGUA, 2014). In Gómez Palacio, Durango there are 11 WWTP, of which two stand out for their treatment capacity, with 500 L s^{-1} through activated sludge and 100 L s^{-1} through stabilization ponds (CONAGUA, 2014). During these processes, a by-product called residual sludge is generated.

The sludge is subjected to microbiological stabilization processes, among them are mechanical (thermal, sun or air drying), chemical (addition of lime or aluminum) and biological (anaerobic or aerobic digestion) (Rigby *et al.*, 2016).

The type of stabilization management limits in some cases the availability of nutrients (Smith *et al.*, 1998; Morris *et al*., 2003; Pu *et al.*, 2008). In the search to reduce the negative effects to the environment due to exposure to residual sludge, there are various alternatives for its disposal, from depositing it in sanitary landfills, incinerating it or using it in agricultural production (Figueroa-Viramontes *et al*., 2010).

Applying biosolids to agricultural soils is the most economical and ecological disposal method due to the recycling of organic matter (OM) and nutrients such as nitrogen (N), phosphorus (P) and potassium (K) (Kumar *et al*., 2014). The degree of humification of OM and the state of mineralization of nutrients affects soil fertility and crop development (Pritchard *et al*., 2010; Lu *et al*., 2012).

After OM, N is the most abundant component in biosolids, this element is also the nutrient most demanded by crops. N mineralization is the biological process by which organic N is transformed into inorganic forms such as ammonium (NH_4^+), nitrate (NO_3^-) and nitrite (NO_2^-), which makes it available for plants and microorganisms in the soil (Bettiol and Camargo, 2008).

Various studies have shown that the use of biosolids has a positive effect on crops. Flores-Felix *et al*. (2014) obtained a higher dry matter (DM) yield with the application of biosolids, with respect to the use of synthetic fertilizer. Shaheen and Tsadilas (2013) reported that the application of biosolids increases biomass in canola.

However, the excessive use of biosolids can generate environmental problems, hence the need to make a rational use of them. One of the criteria used to calculate the dose of biosolids to apply is to evaluate the amount of N available for the crop (NDC) (Silva *et al*., 2013). However, the United States Environmental Protection Agency (US-EPA) establishes that the application rates of biosolids, expressed in kg ha^{-1} of N, should not exceed the requirement of N per cultivation (RNC) (USEPA), 1994)

The NDC from biosolids can be estimated using different methodologies (Mamo *et al*., 1999; Torstensson and Aronsson, 2000; Pu *et al*., 2008). One of the methods is from the fertilizer equivalence; this is calculated based on the response of the crop (in terms of yield or N extraction) to increasing doses of N in the biosolids and the inorganic N of the fertilizer (Barbarick and Ippolito, 2007).

Castor oil plant (*Ricinus communis* L.) is a species of the *Euphorbiaceae* family of economic importance due to its oil content. The oil from its seed can reach a yield of up to 64.5% (González-Chávez *et al*., 2015).

The ecological adaptation that *R. communis* has to biotic and abiotic factors (Bauddh and Singh, 2012) makes it an attractive species, particularly for areas with low water availability. An important factor is that the cultivation of Castor oil plant is industrial and does not enter the trophic chain (Rajkumar and Freitas, 2008) so its production does not compete for food.

Although it is important to note that in the stabilization of the raw sludge used to produce biosolids, the availability of N and other nutrients can affect the information on NDC through the use of biosolids is scarce; therefore, the objective of this study was to determine the availability of N for the cultivation of Castor oil plant (*Ricinus communis* L.) based on two types of biosolids.

Materials and methods

Location of area of study

The experiment was carried out in the facilities of the National Center for Disciplinary Research on Water, Soil, Plant, Atmosphere (CENID-RASPA), of the National Institute of Forestry, Agricultural and Livestock Research (INIFAP), located in the City of Gómez Palacio, Durango, Mexico. The climate of this region corresponds to BWhw, it is characterized by being dry or desert and average annual rainfall of 240 mm, the average annual temperature in the shade is 25 °C (Santamaría-César *et al*., 2006).

The biosolids used were collected from two treatment plants in the City of Gómez Palacio Durango, which work with two different treatment processes, one of them operates with oxidation ponds, while the second has an activated sludge process. Both biosolids were characterized according to NOM-004-SEMARNAT-2002, based on this standard they were classified as excellent for the content of heavy metals and class C for the content of pathogens (Maciel-Torres *et al*., 2015), which allows them to be used in the agricultural sector, either as organic fertilizer or as a soil improver.

Development of the experiment

The experiment was established in 24 L capacity plastic pots, under a greenhouse where the necessary climatic variables were controlled. The Castor oil plant seeds used were variety K 8SS, evaluated in the Valle del Guadiana Experimental Field of the INIFAP, in the state of Durango.

The evaluated treatments consisted of three sources of N and three doses of each, in addition to a control without N. The sources of N were: ammonium sulfate (SA), biosolids from oxidation ponds (BLO) and biosolids from activated sludge (BLA). The SA used contained 21% nitrogen and 24% sulfur. The nutritional characterization of the biosolids is presented in Table 1.

Table 1. Chemical characterization of the biosolids used.

Parameter	pH	EC (dS m^{-1})	OM (%)	P (%)	NTK (%)	Cu (mg kg^{-1})	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})
BLO	7.56	2.53	38.01	0.58	1.23	184.43	4269.5	732.26	283.93
BLA	7.53	3.65	56.83	1.6	2.24	130.6	3579.16	955.7	148

Biosolids from oxidation ponds (BLO); activated sludge biosolids (BLA); electrical conductivity (EC); organic matter (OM); and total Kjeldahl nitrogen (NTK).

The doses of N were 0, 3.79, 5.65 and 7.52 g pot^{-1}, which correspond to 67%, 100% and 133% of the N requirement of the crop (RNC), to calculate the RNC a dose of 80 kg ha^{-1} to cover 100 of the RNC (Rico-Ponce *et al*., 2011) and considering 40% efficiency because it is a sandy soil; that is, the dose of N at 100% of the RNC was 200 kg ha^{-1}.

In Table 2, the amounts of fertilizer and biosolids applied per treatment are recorded. Sowing was carried out in a greenhouse in humid soil at field capacity (CC), three seeds were deposited per pot and when reaching 20 to 25 cm, one plant per pot was left. The soil used in the present work presented a sandy-loamy texture, an inorganic N of 49.18 mg kg^{-1}, pH of 8.74 and electrical conductivity (EC) of 0.66 dS m^{-1}. The harvest was carried out 113 days after sowing.

Table 2. Fertilizer and biosolids dose applied to Castor oil plant (*Ricinus communis* L.).

Source of N	Concentration of N (%)	Nitrogen (g pot^{-1})		
		3.79	5.65	7.52
SA	20.5	18.5	27.6	36.7
BLO	1.23	308	459.7	611.5
BLA	2.25	168.4	251.3	334.3

The treatments were arranged in a randomized block experimental design with four repetitions. The blocking factor was the luminosity, due to the fact that a building attached to the greenhouse managed to shade it for part of the day. The experimental unit was a pot.

Variables evaluated

The plants were divided into the different organs: root, stem, leaf and seed, which were washed with distilled water to eliminate possible contaminants. Subsequently, the plant tissues were left to air dry and finally in a forced air oven at 65 °C, until reaching constant weight to estimate the total dry matter (DM).

The evaluation of the concentration of total N in the different organs was carried out by the combustion method (TruSpec CHN, LECO) starting from the grinding to a 1 mm sieve (Thomas Wiley mill, Mini-Mill model, with integrated sieve). Nitrogen extraction by the plant was calculated by multiplying the dry matter by the total nitrogen concentration in the plant (Barbarick and Ippolito, 2007).

To estimate the NDC in the biosolids in relation to the SA, the fertilizer equivalence dose (DEF) method was used, according to Motavalli *et al*. (1989); Cusick *et al*. (2006); Barbarick and Ippolito, (2007). Regression analyzes were performed between the doses of N applied as chemical fertilizer or as biosolids versus the N extracted by the crop; quadratic equations were selected because they presented a higher value of R^2 (Cusick *et al*., 2006).

With the equations obtained, the value of N extracted in the treatments with biosolids at the dose of RNC= 100% (5.655 g pot^{-1}) was calculated; then the DEF value was calculated, which is the dose of fertilizer that produces the same value of N extracted as a given dose of total N in biosolids. The NDC from biosolids is obtained by dividing the total N dose of biosolids by the N dose of the fertilizer, which produces the same value of N extracted by the crop.

Analysis of data

The data were analyzed with the SAS University Edition statistical package, using the Anova test and orthogonal contrasts, to define the comparison of means between nitrogen sources.

Results

The dry matter (DM) showed a significant difference between the control and the nitrogenous treatments ($p< 0.01$); however, when comparing the type of fertilizer, the statistical difference was not representative (Table 3).

Table 3. Means and orthogonal contrasts, of dry matter, in the different organs of Castor oil plant (*Ricinus communis* L.) in response to doses of ammonium sulfate (SA), oxidation lagoon biosolids (BLO) and activated sludge biosolids (BLA).

Description	Dose N (g pod^{-1})	Dry matter (g plant^{-1})				
		Root	Stem	Leaf	Seed	Total
Control	0	2.21	5.04	8.62	6.07	21.9
SA	3.79	4.22	7.32	17.6	10.6	39.8
	5.65	5.12	9.54	15.8	7.17	37.6
	7.52	5.88	8.24	13.1	12.8	40
Mean		5.07	8.36	15.5	10.2	39.1
CV		16.38	13.31	14.66	27.79	3.36
BLO	3.79	3.44	6.45	13.3	10.1	33.2
	5.65	3.26	6.35	15.6	9.27	34.4
	7.52	4.02	6.99	17.1	9.62	37.7
Mean		3.57	6.6	15.3	9.65	35.1
CV		11.11	5.21	12.51	4.08	6.62
BLA	3.79	4.23	8.7	12.5	9.82	35.2
	5.65	4.16	8.39	15.7	10.7	39
	7.52	4.47	7.36	14.1	11.6	37.5

Description	Dose N (g pod^{-1})	Dry matter (g plant^{-1})				
		Root	Stem	Leaf	Seed	Total
Mean		4.29	8.15	14.1	10.7	37.2
CV		3.79	8.63	11.47	8.25	5.06
Control *vs* rest		*	*	*	*	**
SA *vs* BLO+BLA		ns	ns	ns	ns	ns
SA *vs* BLO		*	ns	ns	ns	ns
SA *vs* BLA		ns	ns	ns	ns	ns
BLO *vs* BLA		ns	ns	ns	ns	ns

ns= non-significant difference; *= $p\leq 0.05$; **= $p\leq 0.01$).

The highest percentage of DM based on the different organs of the plant corresponds to the leaves, with a variation of 33 to 45%, followed by the seed (from 19 to 32%), with the root being the organ with a lower proportion of DM (9.5-15%) (Figure 1).

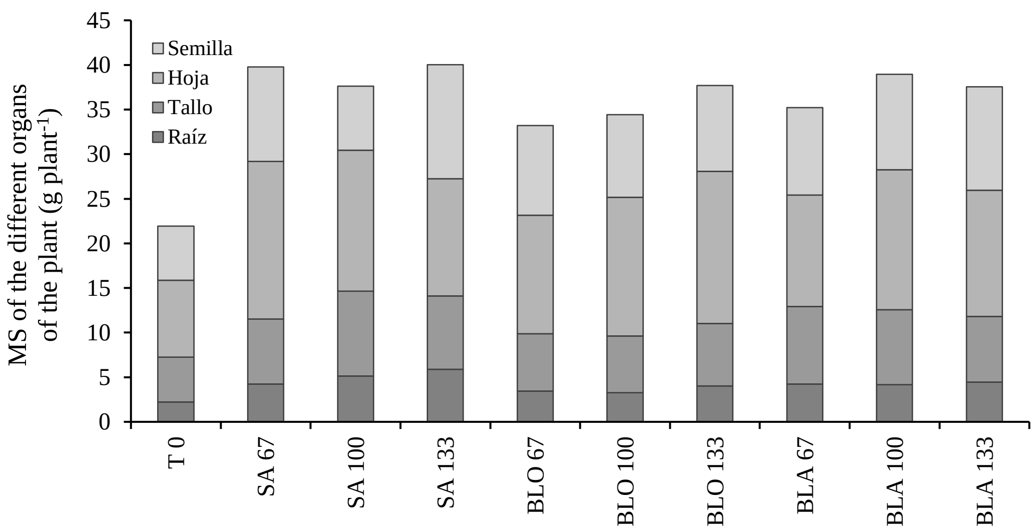

Figure 1. Dry matter of the different Castor oil plant organs, in the different treatments with ammonium sulfate (SA), oxidation lagoon biosolids (BLO) and activated sludge biosolids (BLA) at different doses (0, 67, 100 and 133% of the N requirement of the crop).

A quadratic behavior of the DM yield was observed at the doses of SA (R^2= 0.6), of BLA (R^2= 0.7) and of BLO (R^2= 0.51). With the regression equations ($y= ax^2 + bx + c$) the inflection point that corresponds to the maximum value (VM) of DM can be estimated, using the formula: VM= b/2a. At a N dose of SA of 6.13 g pot^{-1}, a maximum DM value of 40.1 g plant^{-1} was obtained, while the maximum DM value (38.9 g plant^{-1}) in the BLA was obtained at a dose of N of 7.29 g pot^{-1}. On the other hand, a dose of N of the BLOs of 9.57 g pot^{-1} would have to be applied to obtain 38.1 g plant^{-1} of DM (Figure 2).

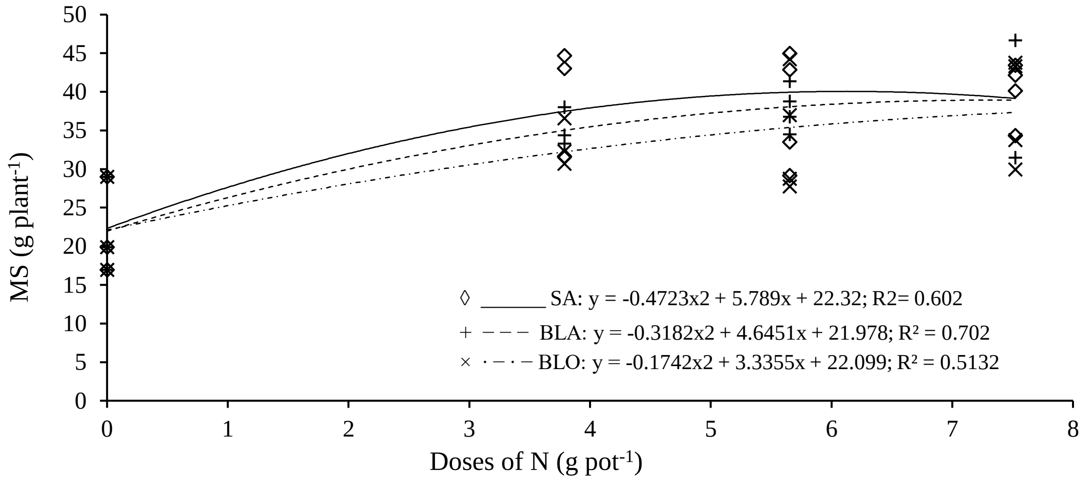

Figure 2. Response of total dry matter (DM) to doses of ammonium sulfate (SA) and activated sludge biosolids (BLA) and oxidation pond biosolids (BLO).

In root, stem, leaf and seed the concentration of N was statistically different between the control group and the rest of the treatments from the orthogonal contrasts performed. The same behavior was observed between the chemical fertilizer and the biosolids for root, stem and leaf ($p< 0.001$), while a $p> 0.05$ was obtained in the seed; however, this organ presented a significant difference between types of biosolids (Table 4).

Table 4. Orthogonal means and contrasts, nitrogen (N) and nitrogen extracted in the different Castor oil plant organs in response to the different treatments.

Description	Dose N ($g\ pot^{-1}$)	Concentration of N				N extracted				
		Root	Stem	Leaf	Seed	Root	Stem	Leaf	Seed	Total
		% (DM)				($g\ plant^{-1}$)				
Control	0	1.19	0.42	1.51	2.67	0.03	0.02	0.13	0.16	0.33
SA	3.79	1.77	1.74	2.81	4.03	0.07	0.13	0.49	0.43	1.12
	5.65	1.9	1.98	2.9	3.87	0.1	0.19	0.46	0.28	1.04
	7.52	1.83	1.97	2.95	3.79	0.11	0.16	0.39	0.48	1.14
Mean		1.83	1.9	2.88	3.89	0.09	0.16	0.44	0.4	1.1
CV		7.17	2.42	3.11	3.11	22.3	19.86	11.67	26.51	5.08
BLO	3.79	1.52	0.6	1.64	3.36	0.05	0.04	0.22	0.34	0.64
	5.65	1.56	1.06	1.98	3.86	0.05	0.07	0.31	0.36	0.78
	7.52	1.59	1.11	1.9	4	0.06	0.08	0.32	0.39	0.86
Mean		1.56	0.92	1.84	3.74	0.05	0.06	0.28	0.36	0.76
CV		2.26	30.96	9.63	9.06	10.83	33.55	20.04	7.64	14.65
BLA	3.79	1.36	0.68	1.65	3.66	0.06	0.06	0.21	0.36	0.68
	5.65	1.57	0.82	1.6	4.35	0.07	0.07	0.25	0.46	0.84
	7.52	1.56	1.06	2.02	4.24	0.07	0.07	0.27	0.49	0.91

Description	Dose N (g pot $^{-1}$)	Concentration of N				N extracted				
		Root	Stem	Leaf	Seed	Root	Stem	Leaf	Seed	Total
		% (DM)				(g plant^{-1})				
Mean		1.5	0.85	1.76	4.08	0.07	0.07	0.24	0.44	0.81
CV		7.92	22.67	12.94	9.07	8.66	11.84	13.74	15.61	14.56
Control *vs* rest		*	**	**	**	*	*	**	*	**
SA *vs* BLO+BLA		*	**	**	ns	*	**	**	ns	**
SA *vs* BLO		*	**	**	ns	*	**	**	ns	**
SA *vs* BLA		ns	**	**	ns	*	**	**	ns	**
BLO *vs* BLA		ns	ns	ns	*	ns	ns	ns	ns	ns

ns= non-significant differences; *= $p \leq 0.05$; **= $p \leq 0.001$; SA= ammonium sulfate; BLO= biosolids from oxidation ponds; BLA= activated sludge biosolids; CV= coefficient of variation.

The total nitrogen extracted by the Castor oil plant tree showed a significant difference between the control and the treatments, as well as between the SA and both biosolids ($p < 0.01$); however, between types of biosolids it was not statistically different according to the orthogonal contrast analysis (Table 2). In general, the highest amount of nitrogen was found in the seed of the evaluated treatments (Figure 3).

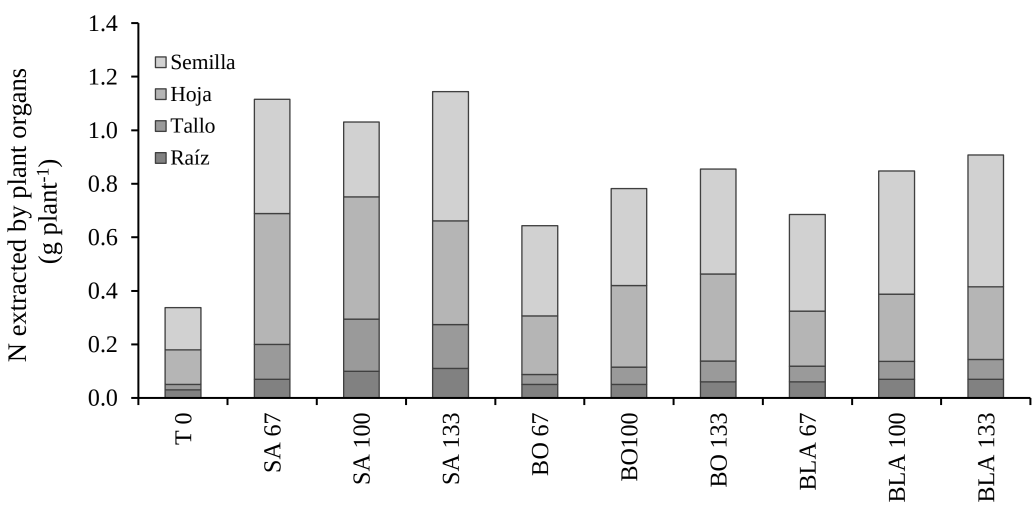

Figure 3. Distribution of N extracted in the different organs of Castor oil plant (*Ricinus communis* L.) in the different treatments with ammonium sulfate (SA), biosolids of oxidation lagoons (BLO) and biosolids of activated sludge (BLA) at different doses (0, 67, 100 and 133% of the N requirement of the culture).

On the other hand, nitrogen extraction showed a quadratic response as a function of the N applied (Figure 4), both with chemical fertilizer ($R^2 = 0.75$), with BLA ($R^2 = 0.91$) and with BLO ($R^2 = 0.71$). To estimate the NDC, first the N extracted in the treatments of 100% of the RNC with biosolids is calculated, using the regression equations; with BLA a value of 0.82 g plant^{-1} was obtained, and with BLO it was 0.77 g plant^{-1}. Subsequently, the DEF was calculated, which in the case of BLA was 2.28 g pot^{-1}, while with BLO it was 1.97 g pot^{-1}. The NDC obtained with the previous values was 40 and 37% for the BLA and BLO, respectively.

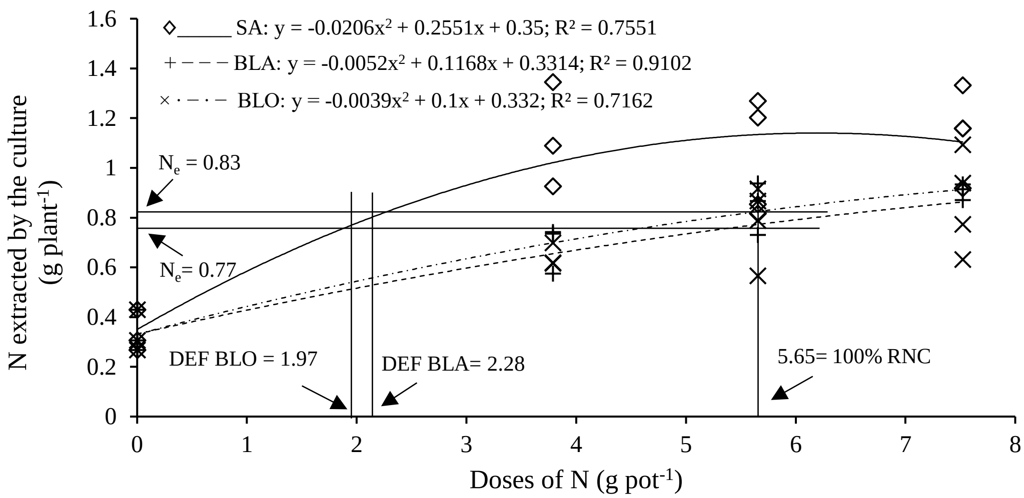

Figure 4. Nitrogen extracted (Ne) by the cultivation of Castor oil plant (*Ricinus communis* L.) and equivalent dose of fertilizer (DEF) of biosolids from oxidation lagoons (BLO) and biosolids from activated sludge (BLA).

Discussion

The range of seed dry matter was 19-32%, which is similar to that reported by Reddy and Matcha (2010), who indicate a percentage of dry matter in Castor oil plant seed of 26.4%. These authors found that a higher proportion of biomass is found in the stem of the plants, while in this work the highest proportion was recorded in leaves (Table 3).

The dry matter of the seed increased by 58, 91 and 110% in the highest N dose with respect to the unfertilized control, when using BLO, BLA and SA, respectively. Similar results were recorded in the work carried out by Reddy and Matcha (2010), in the high dose of applied fertilization. DM production increased as a function of the N dose; it was even observed that, in the highest treatment of the different sources of N, the yield response curve tends to decrease and adjust to a quadratic regression; this is a typical response to increasing doses of N (González-Torres *et al*., 2009) or organic fertilizers (López-Calderón *et al*., 2015).

When this type of response is observed, it is possible to estimate the dose of N to maximize the yield, which corresponds to the inflection point of the curve, in the case of ammonium sulfate, the maximum DM value of 40.1 g plant^{-1} is obtained with 7.29 g pot^{-1} of N, while with BLA and BLO the dose required to obtain the maximum yield was 7.29 and 9.57 g plant^{-1}, respectively. Varvel *et al*. (2007) use these quadratic regression functions to generate recommended fertilization doses, considering the requirement of N to obtain 90 to 95% of the maximum relative yield.

N is the main nutrient for seed and forage production (Mengel *et al*., 2006). Regarding the extraction of N by Castor oil plant, in the present work a greater response to the application of SA was observed. In other similar studies with biosolids, it has been registered that there is no significant difference between the chemical fertilizer and the biosolids used in a cultivation of *Avena sativa* (Flores-Félix *et al*., 2014).

Nitrogen absorption and extraction tends to decrease with high fertilization rates (Motavalli *et al*., 1989), so the curves have a quadratic behavior. The estimated values of NDC are similar to those reported by some authors. Rigby *et al*. (2010) investigated the NDC from three types of biosolids applied to an acid sandy soil, in a Mediterranean climate in western Australia; the values obtained were 65.1%, 63.9% and 39.4% of NDC in biosolids stabilized with lime, biosolids treated with aluminum ($KAl(SO_4)_2 12H_2O$) and anaerobically digested dehydrated biosolids, respectively.

The latter was similar to the BLA that presented a NDC of 40.4%. On the other hand, the BLO showed a NDC of 34.9%, in contrast to that reported by Cogger *et al*. (2004), who determined that the availability of nitrogen from biosolids from lagoons was presented in a range of 8 to 25%. The availability of N for its absorption by cultivation in soils improved with biosolids depends on the forms of N, inorganic N content and the availability of organic N present.

It should be mentioned that the NDC is influenced by various factors such as the type of wastewater treatment, the characteristics of the water to be treated, the type of soil to which it was applied, as well as the type of treatment of the sludge that originate the final biosolids (Al-Dhumri *et al*., 2013; Rigby *et al*., 2016). The usefulness of knowing the NDC in organic fertilizers such as biosolids is that it allows estimating application doses according to the N requirement of the crops, thereby avoiding excessive applications and reducing the risks of environmental contamination.

Conclusion

The application of N from a chemical source and biosolids generated a quadratic response of the dry matter as a function of the dose of N applied. Based on the fertilizer equivalency method, the availability of N for Castor oil plant was 40.4 and 34.8% for BLA and BLO, respectively. Although the nitrogen extraction was higher in the SA treatments, the dry matter was similar between the N sources, which indicates that it is possible to substitute the chemical fertilizer for the biosolids.

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