Nitrogen and phosphorus uptake efficiency in Indian mustard cultivated during three growth cycles in a copper contaminated soil treated with biochar

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ABSTRACT: *Biochar has been used worldwide as an efficient soil amendment due to its beneficial interaction with soil particles and nutrients; however, studies on the effect of biochar on the availability of nutrients such as N and P in tropical soils are still missing. The objective of the study was to evaluate the effect of different types and doses of biochars on the concentration and uptake of N and P in Indian mustard plants (Brassica juncea L.) grown in a Cu contaminated soil during three successive growth cycles. The greenhouse experiment was set up as randomized block design in a 3x3 factorial scheme, with 3 types of biochars (coconut shell, orange bagasse and sewage sludge) and three rates of application (0, 30 and 60t ha⁻¹), and 4 replicates. Biochar increased plant growth by approximately 30 to 224%; however, the orange bagasse biochar was the most effective. Biochar reduced plant N concentration in approximately 15-43%, regardless of the rate of application, indicating the need to carefully adjust N fertilization. In the last growth cycle, biochar from coconut shell and orange bagasse improved the N uptake efficiency suggesting a better amelioration effect with ageing in soil. Biochar did not affect P nutrition in Indian mustard to a great extent; however, it significantly decreased the N:P ratio in the plant.*
Key words: *biochar, pyrogenic carbon, copper contamination, plant nutrition, Brassica juncea.*

Eficiência de absorção de nitrogênio e fósforo em mostarda indiana cultivada por três ciclos sucessivos em um solo contaminado com cobre e tratado com biocarvão

RESUMO: *O biocarvão tem sido usado mundialmente como um eficiente insumo agrícola devido à sua interação benéfica com as partículas e os nutrientes do solo. Contudo, seu efeito na disponibilidade de nutrientes como N e P em solos tropicais tem sido pouco investigado. O objetivo do estudo foi avaliar o efeito de diferentes tipos e doses de biocarvão na concentração e na eficiência de absorção de N e P em plantas de mostarda indiana (Brassica juncea L.) cultivadas em solo contaminado com cobre, em três ciclos sucessivos de cultivo. O estudo foi desenvolvido em delineamento de blocos casualizados, em esquema fatorial 3x3, em casa de vegetação, com três tipos de biocarvão (casca de coco, bagaço de laranja e lodo de esgoto) e três doses (0, 30 e 60t ha⁻¹). Todos os biocarvões aumentaram o crescimento das plantas, com variação de 30 a 224%. No entanto, o biocarvão de bagaço de laranja foi o mais eficiente. A presença de biocarvão reduziu a concentração de N nas plantas em torno de 14 a 43%, independente da dose aplicada, indicando a necessidade de monitoramento mais cuidadoso da fertilização nitrogenada. Os biocarvões de casca de coco e bagaço de laranja melhoraram a eficiência da planta na absorção de N no terceiro ciclo de cultivo, indicando melhor efeito com o tempo de contato como o solo. O uso de biocarvão teve pouca influência na nutrição fosfatada na mostarda indiana, mas diminuiu significativamente a relação N:P.*
Palavras-chave: *biocarvão, carbono pirogênico, contaminação com cobre, nutrição de planta, Brassica juncea.*

INTRODUCTION

Use of biochar as soil amendment has been considered as an environmentally friendly strategy to improve soil fertility while reducing negative impacts caused by the inappropriate disposal of organic wastes. Effect of biochar on soil fertility is believed to be related to its high porosity, surface area and CEC, which increase the soil retention capacity for water and nutrients. However, many other factors such as the biochar liming effect and the presence of a variety of oxygenated functional groups make it difficult to predict all the benefits of biochar to plant growth and productivity (NGUYEN et al., 2017), specially the

effect of biochar on the behavior of N and P in the soil and in the plant (KAMMANN et al., 2015; DEENIK & COONEY, 2016).

Nitrogen and P are very important plant macronutrients and their availability in soil as well as their interaction limits plant growth (GROOT et al., 2003). Studies about the effect of biochar on the availability of N in soil have generated many contradictory results varying from positive, negative and even no effect (CLOUGH et al., 2013; RAHMAN et al., 2013; NGUYEN et al., 2017). The same different outcomes are observed during the evaluation of the effects of biochar on plant N absorption (SAARNIO et al., 2013). Additionally, biochar has also been related to changes in the P cycle in highly weathered acidic soils (ABDELHAFEZ et al., 2017), where the availability of P is mostly controlled by the presence of reactive Al and Fe surfaces that present high affinity for P at low soil pH, causing P to be chemically adsorbed and leading to low agronomic P use efficiency (ANTONIADIS et al., 2015). In that case, the liming effect of biochar to certain extent can increase P availability by reducing P adsorption (VANEK & LEHMANN, 2015).

Plant growth in metal contaminated soils can be seriously impaired not only by high concentrations of heavy metals but also by availability of nutrients and nutritional status, including heavy metal tolerant ones such as Indian mustard (MOURATO et al., 2015) that are often used as food crop as well as to remediate contaminated soils (RODRÍGUEZ-VILA et al., 2015). Therefore, the objective of this study was to evaluate the concentration of N and P in plant tissue as well as N and P use efficiency in Indian mustard plants cultivated in a copper contaminated soil and treated with different types and doses of biochar, during three successive growth cycles.

MATERIALS AND METHODS

Soil and biochar characterization

Soil was collected in the 0-0.20m layer from a fallow field at the Universidade Federal de Sergipe (UFS) experimental station, Northeast Brazil (about S 10º 55' 46"; N 37º 06' 13"). The soil was classified as Argissolo Amarelo (SANTOS et al., 2013), (Ultisol, according to SOIL SURVEY STAFF (2014)) and presented the following characteristics: pH: 4.64, organic carbon: 11.6g kg^{-1}, CEC: 1.88cmol kg^{-1}, P: 1.82mg kg^{-1}, K: 25.4mg kg^{-1}, total Cu: 100mg kg^{-1}. Physical properties were: Sand: 72%, Silt: 13%, Clay: 15% (DAY, 1965).

The biochars were produced from coconut shell, orange bagasse and sewage sludge in a slow pyrolysis reactor, at 500ºC. Biochar was ground, sieved to 2mm particle size and evaluated through proximate analysis (Ash, volatile matter and fixed C), and ultimate analysis (C, N, H and O) according to the American Society for Testing and Materials (ASTM) D1752-84. Elemental composition of C, H, and N was determined using an elemental analyzer. Cation exchange capacity was determined by ammonium acetate method (THOMAS, 1982). Biochar pH and electrical conductivity were also determined. Biochar characteristics are presented in table 1.

Experimental set up

The experiments were carried out in a greenhouse environment at a temperature of 28ºC and as a completely randomized design in a 3x3 factorial scheme, with 4 replicates. Treatments were three types of biochar (coconut shell-CSB; orange bagasse – OBB; and sewage sludge – SSB) and three rates of application (0, 30 and 60t ha^{-1}). Experimental units were comprised of 5L plastic pots. Soil was spiked with copper sulfate three months prior the experiment. Biochar (37,5g or 75g) was mixed with the contaminated soil (3.5kg), transferred to pots, watered to field capacity and incubated during one week before planting in order to equilibrate. One healthy Indian Mustard seedling with four leaves was transferred to each pot and allowed to grow for 60 days. Plants were watered throughout the study to keep the soil at approximately 70% of its field capacity. Indian mustard plants were cultivated for three successive growth cycles (April to June; June to August; August to October, 2016). At the beginning of each growth

Table 1 - Characteristics of biochars.

Biochar	VM	Ash	FC	C	N	P	pH	CEC
	-- % --							cmolc kg^{-1}
CSB	15.8	10.8	67.2	79.5	0.42	0.02	10.6	69.7
OBB	17.6	16.1	58.7	72.3	2.55	0.52	10.0	63.5
SSB	26.0	36.0	34.1	43.0	6.80	1.06	7.30	22.2

cycle, 1g of mineral fertilizer (osmocote:14-14-14) was applied to each pot.

Before harvesting, chlorophyll content of the mid-section, fully expanded leaves was measured, using a non-modulated fluorimeter OS-30P model (ADC BioScientific Ltd., UK). After each harvesting, plants were washed with tap water, rinsed with DI water and dried in a 60°C oven for approximately 48 hours, weighed for the determination of the dry biomass and ground to pass through a 2mm screen for N and P analysis. Samples were digested in a hot block digestion system, according to the TKN protocol for N determination (BREMNER, 1996). Total P concentration in plant samples was determined in the TKN extracts using the molybdenum blue method (MURPHY & RILEY 1962). Soil samples were collected at the beginning of each growth cycle from each experimental pot, dried, sieved to 2mm screen and analyzed for pH, EC, organic C and concentration of extractable Cu, N, P, K and Na. Soil pH was determined in a 1:5 (w/w) biochar:water ratio after 1.5h shaking in a reciprocating shaker and 1h equilibration. Concentrations of extractable Cu, P, K and Na were determined following extraction with 1% citric acid (WU et al., 2004). Soil plant available N was extracted with 2M KCl. The uptake efficiency of N or P was calculated by dividing N or P uptake by the root biomass dry weight (GOOD et al., 2004).

Statistical analysis

Statistical analysis was performed separately on each crop cycle because the experiments were not run concurrently. Results are reported as the means of four replicates. Data obtained were subjected to two-way Analysis of Variance (ANOVA) for assessing the significance of quantitative changes in the variables as a result of biochar treatments using SISVAR software package (FERREIRA, 2011). When a significant difference was observed between treatments, multiple comparisons were made by the Tukey-test at $P<0.05$.

RESULTS AND DISCUSSION

Effect of biochar in plant biomass

Application of biochar significantly ($P<0.05$) increased plant biomass in all growth cycles (Table 2); however the effects varied with biochar type and rates of application. In the first and third growth cycles, biochar from coconut shell increased plant biomass by approximately 50 and 32%, regardless of the rate of application. In the second growth cycle, the increase was according to the rate of application, being 100% (30t ha^{-1}) and 224% (60t ha^{-1}). The OBB was more effective in increasing plant biomass in all growth cycles, varying from 168% (GC1), 148-191% (GC2, 30t ha^{-1} and 60t ha^{-1}, respectively) and 60%

Table 2 - Biomass and concentration of N and P in the shoot of Indian mustard plants after growing in the copper contaminated soil treated with biochar from coconut shell (CSB), orange bagasse (OBB) and sewage sludge (SSB). Means followed by same letter (uppercase in a row; lowercase in a column) are not significantly different (P < 0.05) for comparisons made within each crop cycle (n = 4 per treatment).

Rate of application	CSB	OBB	SSB	CSB	OBB	SSB	CSB	OBB	SSB
	-----------Growth cycle 1-----------			------------Growth cycle 2--------------			------------Growth cycle 3--------------		
---Plant biomass (g dw plant^{-1}) ---									
0	1.28Ab	1.28Ab	1.28Aa	2.74Ac	2.79Ac	2.79Ab	3.80Ab	3.60Ab	3.60Ac
30	1.97Ba	3.96Aa	1.05Ca	5.48Bb	6.92Ab	7.00Aa	5.09Aa	5.87Aa	5.37Ab
60	1.94Ba	2.92Aa	1.34Ba	8.90Aa	8.13Aa	6.92ba	4.97Ca	5.65Ba	6.80Aa
--Plant N (g kg^{-1})--									
0	27.8Aa	27.8Aa	27.8Aa	28.0Aa	28.0Aa	28.0Aa	46.9Aa	46.9Aa	46.9Aa
30	23.4Bb	21.3Bb	29.1Aa	18.3Ab	15.1Ab	16.8Ab	33.9Ab	34.5Ab	32.6Ab
60	23.9Ab	19.7Bb	29.5Aa	15.2Ab	16.5Ab	17.9Ab	37.2Ab	31.9Bb	34.4ABb
---Plant P (g kg^{-1}) --									
0	3.14Ab	3.14Ab	3.14Aa	3.88Aa	3.88Aa	3.88Aa	5.20Aa	5.18Aa	5.20Aa
30	5.42Aa	5.04ABa	3.97Ba	3.34Aa	3.98Aa	4.03Aa	4.92Ba	5.93Aa	4.96Ba
a60	3.94Ab	3.04Ab	3.40Aa	3.59Aa	4.40Aa	4.08Aa	5.30Aa	5.72Aa	5.40Aa
---N : P ratio---									
0	7.23Aa	7.23Aa	7.23Aa	8.22Aa	8.22Aa	8.22Aa	9.40Aa	9.07Aa	9.03Aa
30	5.10Ab	3.97Bb	4.65ABb	4.74Bc	4.29Bc	6.88Ab	7.50Ab	5.83Bb	6.70ABb
60	4.36Ac	3.83Ab	4.42Ab	6.37Bb	6.50Bb	8.38Aa	7.05Ab	5.57Bb	6.41ABb

(GC3). The effect of the SSB on plant biomass was only observed in the GC2 (150%) and GC3 (50% and 90%, for 30t ha^{-1} and 60t ha^{-1}, respectively), confirming the beneficial effect of this biochar as reported by SILVA et al. (2017). Biochar effect in increasing plant growth was probably due to the amelioration effect in the contaminated soil by increasing soil pH and the availability of some nutrients such as P and K which was confirmed in the study of DEENIK & COONEY (2016) when corn cob and sewage sludge biochar was applied to an oxisol. The authors observed that biochars in combination with fertilizer doubled plant growth compared to the control in the first crop cycle produced no significant effect in the second cycle, and more than tripled plant growth for the sewage sludge biochar in the third cycle. In the present study, plants presented poor growth in the first cycle due to a problem in the greenhouse.

Effect of biochar in N nutrition

The increase in plant biomass caused by the presence of biochar was not followed by an increase in the concentration of N in the plant aboveground part, which varied from 15.1 - 46.9g kg^{-1}. Considering that the concentration of N commonly ranges from 23.9 - 55.1g kg^{-1} for plants of the *Brassica* genus, the presence of biochar, especially in the GC2, caused a reduction in N concentration below the lower limit in most plants. In fact, all biochars reduced plant N (Table 2) in approximately 15-43%, regardless of the rate of application, which could be observed by the chlorotic appearance of leaves. Considering that all the plants were equally fertilized with N, the yellow discoloring of the leaves observed in the treatments with biochar is supposed to be related to the effect of biochar on the fate of N and not to the effect of the high Cu concentration in the contaminated soil. Therefore, N fertilization in soils treated with biochar should be a concern. Reduction in plant N concentration due to biochar was also confirmed by the reduction in the chlorophyll content (Table 3) and could have been a result of the improved growth and consequently increased demand for this essential macronutrient. ARGENTA et al. (2004) also reported good correlations between plant N and Chlorophyll content in maize plants.

Additionally, it was reported that during the ageing of biochar in the soil, NO_3-N chemisorption through H-bonding may be enhanced because biochar becomes more hydrophilic (HAMMES & SCHMIDT, 2009); however, in the present study, the evaluation of N availability through KCl extraction showed little influence of the biochars (Table 4), except in the OBB treatment which significantly decreased extractable N. This effect could also be related to the increase in pH when the OBB was applied to soil (Table 4), leading to N loss through volatilization (NGUYEN et al., 2017). Therefore, different biochars may likely influence different mechanisms of N interaction in soil for which more investigation is needed.

The evaluation of the N uptake efficiency (NUE) showed a huge variation in the treatments among growth cycles (Figure 1). In the first GC, when biochar was recently incorporated to the soil,

Table 3 - Chlorophyll A, Chlorophyll B and ChloA/ChloB ratio of Indian mustard plants after growing in the copper contaminated soil treated with biochar from coconut shell (CSB), orange bagasse (OBB) and sewage sludge (SSB). Means followed by same letter (uppercase in a row; lowercase in a column) are not significantly different (P < 0.05) for comparisons made within each crop cycle (n = 4 per treatment).

Rate of application	CSB	OBB	SSB	CSB	OBB	SSB
	-----------------------Growth cycle 1------------------------			-----------------------Growth cycle 2------------------------		
--Chlorophyll A (SPAD) --						
0	28.3Aa	28.3Aa	28.3Aa	27.9Aa	27.9Aa	27.9Aa
30	27.1Aa	27.2Aa	29.1Aa	24.0Ab	23.2Ab	23.8Ab
60	26.1Aa	20.5Bb	29.5Aa	22.5Ab	23.7Ab	21.6Ab
--Chlorophyll B (SPAD) --						
0	11.1Aa	11.1Aa	11.0Aa	10.0Aa	10.0Aa	10.0Aa
30	9.42ABa	8.12Bb	11.1Aa	6.57Ab	6.90Ab	8.00Ab
60	11.0Aa	6.22Bb	11.5Aa	7.15Ab	7.02Ab	6.52Ab
--Chlorophyll A: Chlorophyll B ratio--						
0	2.55Aab	2.55Ab	2.55Aa	2.81Ab	2.81Aa	2.81Aa
30	2.88Ba	3.34Aa	2.64Ba	3.70Aa	3.38ABa	3.00Ba
60	2.40Bb	3.32Aa	2.57Ba	3.20Aab	3.40Aa	3.28Aa

Table 4 - Extractable concentrations of P, N, K and Na, pH and EC in the copper contaminated soil treated with biochar from coconut shell (CSB), orange bagasse (OBB) and sewage sludge (SSB). Uppercase letters compare means among different biochars at the same dose. Lowercase letters compare means among different doses at the same biochar type ((P < 0.05) (n = 4 per treatment).

Biochar type	Rate of application	P	N	K	Na	pH
		--mg kg^{-1}--				
CSB	0	44.1Aa	2.96Aa	50.1Ac	0.09Ac	4.79Abc
	30	40.1Ba	2.57Aa	170Ab	120Ab	5.60Bb
	60	44.8Ca	3.38Aa	338Ba	262Aa	6.50Aa
OBB	0	46.4Ab	2.96Aa	50.1Ac	0.09Ab	4.79Ab
	30	67.0Aa	1.70Aab	302Bb	20.7Ba	6.26Aa
	60	72.0Ba	1.60Bb	660Aa	28.0Ca	7.06Ba
SSB	0	43.4Ac	2.96Aa	50.1Aa	0.09Ac	4.79Ab
	30	74.7Ab	2.14Aa	59.4Ca	28.7Bb	5.74Ba
	60	97.8Aa	0.65Bb	19.9Cb	82.6Ba	5.84Ca

NUE varied from 0.57 to 0.80g N plant^{-1} g root^{-1}, and only the OBB reduced the plant efficiency in taking up N. The NUE was greatly reduced in the second GC in all treatments, including the control, varying from 0.08 to 0.18g N plant^{-1} g root^{-1}; however, this time all biochars caused reduction in the capacity of the plant to absorb N, except for CSB at 30t ha^{-1} and OBB at 60t ha^{-1}. In the third GC, all biochars increased in approximately 80% the values of NUE, varying from 0.2 to 0.5g N plant^{-1} g root^{-1}, except the SSB when applied at a rate of 30t ha^{-1}.

Effect of biochar in P nutrition

The concentration of P in the shoot varied from 3.40 to 5.93g kg^{-1} (Table 2). Biochar did not affect P nutrition in Indian mustard, except for the CSB and OBB applied at a rate of 30t ha^{-1}, in the first GC, which increased P concentration in the aboveground biomass by approximately 72% and 61%, respectively. These results are not likely related to the concentration of citric acid extractable P in the soil since application of CSB had no effect on P availability, regardless of the increase in soil pH caused by this biochar. In fact, application of OBB and SSB even increased the concentration of extractable P (Table 4), which could be related not only to the increase in soil pH but also to the concentration of P in the biochar (Table 1). A reasonable explanation for the lack of plant response related to P nutrition is the lower P requirement by Indian mustard plants which have higher demand for N.

The plant efficiency in taken up P greatly varied among growth cycles (Figure 1), from 0.09-0.14g P plant^{-1} g root^{-1} (GC1), 0.02-0.03g P plant^{-1} g root^{-1} (GC2) and 0.02-0.10g P plant^{-1} g root^{-1} (GC3). CSB and OBB significantly (P<0.05) increased P uptake efficiency (PUE), regardless of the rate of application. SSB had low influence on the PUE.

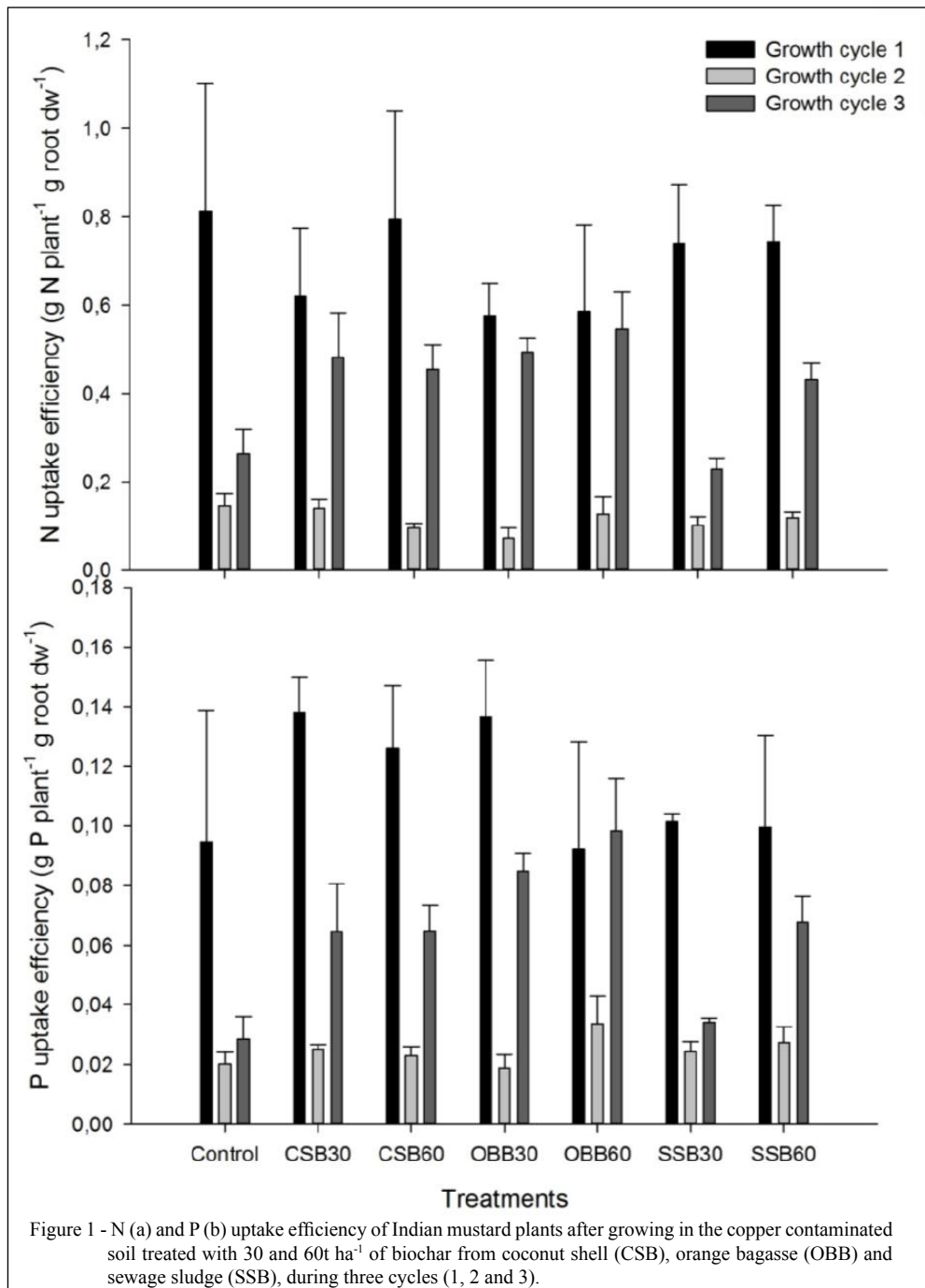*Effect of biochar on the N:P ratio in the plant*

Biochar addition to soil significantly (P<0.05) reduced the N:P ratio in the plants, regardless of the type and rate of application (Table 2). In the control treatment, N:P ratio varied from 7.23 to 9.04 across the three growth cycles whereas in the biochar treatments this variation was from 3.83 to 8.38, with mean values of 5.85, 5.00 and, 6.24 for CSB, OBB and SSB, respectively, with lowest values mostly associated with the OBB. According to GARRISH et al. (2010), the N:P ratio is an effective tool to evaluate the availability of these nutrients to plants; values < 14 indicates N limitation whereas values > 16 are a sign of P restriction to plant productivity. Therefore, our values confirmed N limitation in plants treated with biochar.

CONCLUSION

All biochars increased plant growth and reduced plant N concentration; however, the most effective was the orange bagasse biochar, indicating the need for a more cautious management of the N fertilization. Biochar increased plant N uptake efficiency which could explain the improved plant growth.

Even though biochar from orange bagasse and sewage sludge increased the availability of P in soil, no biochar influenced plant P nutrition, regardless of the increase in pH

Figure 1 - N (a) and P (b) uptake efficiency of Indian mustard plants after growing in the copper contaminated soil treated with 30 and 60t ha^{-1} of biochar from coconut shell (CSB), orange bagasse (OBB) and sewage sludge (SSB), during three cycles (1, 2 and 3).

of this acidic soil. This was probably related to the plant species itself and its lower demand for P than for N.

Considering that the plants had poor growth in the first cultivation cycle and; therefore, should not be taken into account, the effect of biochar on the plant N and P use efficiency seemed to improve as biochar aged in the soil from the second to the third cycle.

CONFLICTS OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

The authors contributed equally to the manuscript.

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