Effect of pellets made of waste materials from the paper industry enhanced with seaweed (*Ulva lactuca* L.) on N mineralization and lettuce production

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ABSTRACT

The need to reduce the use of traditional fertilizers and prevent soil degradation demands the search for new ways of fertilization. The combined application of waste materials (generated by the paper industry) and seaweed may be an alternative source of fertilization. The objective of this study was to evaluate the effect of pellets made of waste materials ash and sludge, produced by the paper industry with the addition of seaweed (*Ulva lactuca* L.) on the production of lettuce (*Lactuca sativa* L.) as an indicator plant and N mineralization in the soil. Two trials were conducted on an Entisol soil. The first trial evaluated the productive response of lettuce to the combined application of pellet+seaweed. Lettuce plants were grown in pots under greenhouse conditions for 3-mo. Three types of pellets, with and without seaweed added, were applied in doses of 10, 20, and 30 t ha^{-1}. N mineralization was measured by 12 wk incubation, evaluating the three types of pellets with and without the addition of seaweed. The results showed that all the combined pellet+seaweed treatments had higher aerial biomass production ($p \leq 0.05$) and increased chlorophyll content. The aerial biomass production reached 4.16 g pot^{-1} and was 7-fold higher than pellet treatments without seaweed, while chlorophyll content was 17% higher ($p \leq 0.05$). In terms of N mineralization, the maximum release was recorded in the Pellet 3+seaweed treatment, reaching 55 mg kg^{-1}. All the treatments with seaweed ($p \leq 0.05$) better higher N mineralization values compared to the treatments without seaweed. Similarly, increased values for potentially mineralizable N and mineralization rate constant were observed in the treatments that included pellets+seaweed and seaweed alone compared to those consisting of pellets alone. The pellets+seaweed amendments resulted in an increase in soil N mineralization and production parameters of lettuce.

Key words: Biomass ash, *Lactuca sativa*, N mineralization, paper industry sludge, seaweed.

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INTRODUCTION

Waste from industrial processes represents a source for the development of new fertilizers for agricultural and forest use. In Chile, 10.4 million tons of industrial solid waste were produced in 2009, out of which 18% came from manufacturing industries, including the paper industry (CONAMA, 2010). In the Biobío Region, the paper industry produced a total of 4961 t of non-hazardous industrial waste, at a rate of 15-30 kg waste per ton of paper produced, including sludge, ash, and wastewater (CORMA, 2012).

Sludge from the paper industry has a high content of organic matter (OM). When applied to the soil as an amendment, it can improve physical soil properties and also increase N, P, and K contents (Camberato et al., 2006). Kumar and Chopra (2014) reported that yield of a *Phaseolus vulgaris* L. crop increased when pulp and paper mill effluents were used as a soil amendment. The results of a study conducted by Ríos et al. (2012) revealed that the application of sludge from the paper industry as an amendment increased OM content, Olsen-P, S-SO_4, Zn, and pH in the soil, although Fe and Mn levels decreased. The results also revealed average values for N levels so that N availability when using this type of sludge has not been reported as an important contribution to the elemental composition of this material, where N is an essential element for the biomass production of plants. The use of wood ash in agricultural soils is not a risk to the environment, unless excessive amounts or only ashes from pure wood waste materials are applied (Demeyer et al., 2001). Ash wood is a source of nutrients, such as P, K, Mg and Ca, except for C and N which volatilize during combustion (Odlare and Pell, 2009). The use of ash in acid soils showed increases in P adsorption of the soil (Seshadri et al., 2013). Ochecova et al. (2014) indicated that wood ash is beneficial to the soil as an amendment, as its application showed increases in nutrients in the soil and plants under study. Sludge and ash may be pelletized to reduce the water content of the sludge and ash surface area, facilitating transport, packaging and soil application (Steenari et al., 1998).

Soil OM by mineralization releases macronutrients and micronutrients depending on the amount applied (Rahman et al., 2013). N mineralization from soil OM indicates the amount of organic N transformed into inorganic N, considering moisture, incubation time and temperature of the soil (Gilmour and Mauromoustakos, 2010).

Marine biota such as seaweed can be also used as soil amendments. In fact, their use as fertilizers has been reported in the coastal areas of Europe, North America and the East (Kornicker, 1961). In Chile,

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decomposed seaweed has been applied to the soil (Buschmann et al., 2005). In many coastal areas, *Ulva* spp. reaches a large amount of biomass in the spring-summer season, even affecting tourism (Stotz, 2007). *Ulvaceae* are characterized by high contents of OM (Aguilar and Guerrero, 2005) whose role in promoting plant yield and seed germination has been described in the literature (Gireesh et al., 2011).

This study hypothesizes that pelletized waste materials from the paper industry can have a better performance as a soil amendment when combined with seaweed (*Ulva lactuca* L.), improving the production of lettuce (*Lactuca sativa* L.) and increasing N mineralization. Therefore, the objective of this research was to evaluate the fertilizing effect of pelletized waste materials from the paper industry combined with seaweed on the production of lettuce and N mineralization in the soil.

MATERIALS AND METHODS

Waste materials from paper industry and seaweed

Waste from paper industry is released in the process of papermaking, which releases both sludge and ash. These two waste materials were used to produce three types of pellets at the Technological Development Unit (UDT) of the University of Concepción. The pellets consisted of different amounts of sludge, ash, and gypsum as an inert binder material. The amounts used were up to 70% ash and 20% sludge (Table 1). The chemical characterization of the sludge, ash and gypsum was conducted according to the methodology described by Sadzawka et al. (2005). Total N, C/N, and OM contents were measured by digestion, while the contents of P, K, Mg, and Ca were determined by atomic emission spectrometry (Perkin-Elmer spectrometer, model 1100B, Phoenix, Arizona, USA). The sludge had an OM content of 51.9% and a good C/N ratio of 15.5, which can be considered as an adequate C source for microbial activity and N mineralization (Table 2). The ash had a high total C content, indicating that there is an incomplete combustion of wood waste materials used. Contents of K and Ca were high, reaching levels considered regular for these types of materials (Table 2).

Seaweed (*Ulva lactuca* L.) was collected at Dichato (36°32' S, 72°56' W), on the coast of the Biobío Region, Chile. This seaweed is found in large quantities from spring to early autumn (Stotz, 2007). The collected seaweed was pre-dried on a sieve in a greenhouse at room temperature, then dried at 60 °C for 10 h in a forced-air stove (UL 543

Table 1. Amount of ash, sludge, and gypsum used to make different types of pellets.

Pellet	Ash	Sludge	Gypsum
		%	
PE1	70	0	30
PE2	63	10	27
PE3	56	20	24

PE1: Pellet 1; PE2: pellet 2; PE3: pellet 3.

Table 2. Chemical characterization of the sludge, ashes, gypsum of the pellets and seaweed (*Ulva lactuca*) used in the trials of lettuce and N mineralization.

Element[1]	Sludge	Ash	Gypsum	Seaweed
N total, %	1.68	0.56	-	3.10
P total, %	0.29	0.25	< 0.01	0.25
K total, %	0.09	2.11	0.20	2.24
Mg total, %	0.14	1.65	0.05	1.43
Ca total, %	0.25	4.99	23.70	0.78
$N\text{-}NO_3$, mg kg^{-1}	0.17	-	-	20.00
MO, %	51.90	-	-	59.40
C total, %	26.00	24.30	-	34.45
C/N	15.50	-	-	11.10

[1]Analytical methods applied by Sadzawka et al. (2005).

H, Blue M, Blue Island, Illinois, USA) and finally ground in a mill provided with a 20-mesh sieve (5KH39QN5525, General Electric, Fairfield, Connecticut, USA). The chemical characterization of seaweed was performed according to the methodology described by Sadzawka et al. (2005), determining the concentrations of C, C/N, OM, N, P, K, Ca, Mg, and $N\text{-}NO_3$. The obtained material had a high content of OM, showing a high potential as an organic amendment. The C/N ratio was low and reached a value of 11.1, while N, K, and Mg concentrations were high (Table 2).

Soil used in the pots and N mineralization

The soil corresponds to an Entisol (Stolpe, 2006) and it was taken at a depth of 0-20 cm in Quillón (36°45' S, 72°25' W), Biobío Region, Chile. The soil was dried at room temperature and sieved to 2 mm size screen (Sandoval et al., 2012). The chemical soil characterization was performed according to the methodology described by Sadzawka et al. (2006). Water pH and OM were measured, while concentrations of macronutrients (P-Olsen, K, Ca, Mg, Na and S) and micronutrients (Fe, Mn, Zn, Cu and B) were also determined. The soil has a sandy texture and a bulk density of 1.3 g cm^{-3}. The chemical analysis of the soil at the beginning of the trial (Table 3) showed low levels of OM, macronutrients (such as nitrates, P, available K and S) and micronutrients. This corresponds to a medium to low level of nutrient availability (Vidal, 2007), which means that it is a good substrate to evaluate the response to the amendments under study.

Table 3. Initial chemical characterization of the soil used in the trial of lettuce and N mineralization.

Parameter[1]	Values
Water pH	6.83
Organic matter, %	2.08
Nitrates ($N\text{-}NO_3$), mg kg^{-1}	3.50
P Olsen, mg kg^{-1}	13.50
K interchangeable, $cmol_c$ kg^{-1}	0.17
Ca interchangeable, $cmol_c$ kg^{-1}	3.93
Mg interchangeable, $cmol_c$ kg^{-1}	0.58
Na interchangeable, $cmol_c$ kg^{-1}	0.07
Available S, mg kg^{-1}	0.10
Fe, mg kg^{-1}	19.60
Mn, mg kg^{-1}	0.40
Zn, mg kg^{-1}	0.50
Cu, mg kg^{-1}	0.50
B, mg kg^{-1}	0.20

[1]Analytical methods applied by Sadzawka et al. (2006).

Effect of pellets and seaweed on the production of potted lettuce

The trial was conducted in pots in a glass greenhouse of the College of Agronomy of the University of Concepción, Chillán. Each pot was filled with 2 kg dry soil and 100 g perlite to increase moisture retention of the sandy soil. A dose of seaweed and pellet was placed in a polyethylene bag and homogenized, and the mixture was then added depending on the treatment. Ten treatments without seaweed and 10 treatments with seaweed (AL_{10}) were evaluated, including three different types of pellets (PE1, PE2, PE3), in doses equivalent to of 10, 20 and 30 t ha^{-1} ($PE1_{10, 20, 30}$, $PE2_{10, 20, 30}$, $PE3_{10, 20, 30}$), plus a control treatment without pellets (T0). The same treatments were repeated with the addition of seaweed (AL_{10}) in doses of 10 t ha^{-1} ($AL_{10}PE1_{10, 20, 30}$, $AL_{10}PE2_{10, 20, 30}$, $AL_{10}PE3_{10, 20, 30}$), including a control (AL_{10}). To obtain equivalent doses of pellets+seaweed, a depth of 0-20 cm of soil per hectare and an apparent density of 1.3 g cm^{-3} were considered. Then 10 lettuce seeds (*Lactuca sativa* L. var. *capitata*) were planted per pot. The trial was maintained at 80% field capacity (FC) controlled on basis by gravimetry and at room temperature for 3-mo.

The initial relative growth rate (RGR) was determined 3 wk after the experiment started by removing two plants from each pot, while plant thinning was conducted 4 wk after the experiment started, leaving two plants per pot. Measurements of aerial biomass (AB), total N (Nt) and chlorophyll content (CL) in leaves were also determined. The RGR was determined as proposed by Hunt (1982):

$$RGR = \frac{[Ln\ (sample2] - [Ln\ (sample1]}{time2 - time1} \qquad [1]$$

where, *RGR* is the relative growth rate, *Ln* is the natural logarithm, *sample 1* is the dry weight of sample 1 at *time 1* and *sample 2* is the dry weight of the sample 2 at *time 2*. This parameter represents the plant efficiency to produce new material in a given time, expressed in amount of DM gained amount of DM already existing per time unit (g g^{-1} d^{-1}).

The amount of lettuce AB was estimated gravimetrically by drying to constant weight at 60 °C in a forced air oven; Nt was determined by the methodology described by Sadzawka et al. (2007) by digestion and manual titration; and CL concentration was measured with a SPAD 502 meter (Minolta, Spectrum Technologies, Chicago, Illinois, USA). The value obtained was expressed in SPAD readings that estimate the relative chlorophyll content in the leaf (Yang et al., 2003).

The experiment was a completely randomized factorial arrangement (2 × 10) with three replicates, with and without addition of seaweed and 10 different combinations of pellet types and doses, including a control treatment consisting of unamended soil.

Assessment of net N mineralization by incubation in pellets with seaweed

A second incubation trial was performed under controlled conditions in an incubator (D-3162, Köttermann GmbH & Co. KG Labortechnik, Uetze, Germany). Amounts of 100 g dry soil with addition of different treatments were placed in plastic containers of 150 mL. Three treatments were applied, using three types of pellets (PE1, PE2, PE3), in doses of 10 t ha^{-1} ($PE1_{10}$, $PE2_{10}$, $PE3_{10}$). These same treatments were repeated with the addition of seaweed (AL_{10}) in doses of 10 t ha^{-1} ($AL_{10}PE1_{10}$, $AL_{10}PE2_{10}$, $AL_{10}PE3_{10}$). Additionally, the following treatments were also included: one treatment consisting of soil alone (T0); one treatment with seaweed alone in a dose of 10 t ha^{-1} (AL_{10}) and one treatment with inorganic fertilization (TF) according to the soil requirements, consisting of 240 kg N ha^{-1} as urea. There was a total of nine experimental treatments in a completely randomized design with three replicates. All treatments and replicates were quintupled to be assessed at five different time periods: 0, 2, 4, 8, and 12 wk.

Samples were incubated aerobically until week 12 at 25 °C. Moisture was maintained at 80% FC and controlled on a weekly basis by gravimetry, according to the methodology used by Hirzel et al. (2010).

Inorganic N (NH_4^+ + NO_3^-) was determined at weeks 0, 2, 4, 8 and 12, considering the three replicates per treatment. Net N mineralization (MNt) was calculated as the inorganic N for each sampling time minus the amount determined at time zero (Hirzel et al., 2010). Soil inorganic N was extracted with 2 M KCl at a 1:5 ratio (Keeney and Nelson, 1982) and analyzed colorimetrically with an autoanalyzer (Skalar, Sanplus, Breda, The Netherlands). A regression analysis was used to determine the N mineralization potential (MP), where it was assumed that N mineralization was a first-order reaction (Tyson and Cabrera, 1993):

$$Nm_t = No\left[1 - e^{(-kt)}\right] \qquad [2]$$

where Nm_t is mineral N accumulated in a specific time (mg kg^{-1}), *No* is potentially mineralizable N (mg kg^{-1}), *k* is the mineralization rate constant, and *t* is the incubation time in weeks.

Statistical analysis

The statistical analysis of data obtained in terms of the production parameters of lettuce and N mineralization was performed with ANOVA, while the effect of means was analyzed by Tukey's test with a confidence level of 95% (α = 0.05). Potentially mineralizable N (*No*) and mineralization rate constant (*k*) were determined using the Gauss-Newton method for nonlinear least squares (Bonde et al., 1988). Data were analyzed using SAS software (SAS Institute, Cary, North Carolina, USA).

RESULTS AND DISCUSION

N mineralization with pellets and seaweed in an Entisol

Net N mineralization (MNt) in response to the incubation of pellets with and without seaweed added in an Entisol soil (for a period of 12 wk) shows that the treatments with different pellets and doses plus 10 t ha^{-1} seaweed had higher MNt ($p \leq 0.05$) compared to the treatments with pellets alone, which occurred in every incubation week (Table 4). The treatment with inorganic fertilizer (TF) presented a high release of N, with higher values ($p \leq 0.05$) than those recorded in all treatments with addition of organic amendments from week 2 to week 8, while the rest of treatments did not reach the levels of mineral N observed with TF. However, treatment $AL_{10}PE3_{10}$ recorded a value at week 12 that was significantly equal to that of TF (Table 4). This means that the amendment made of 10 t ha^{-1} of Pellet 3 plus seaweed released an amount of N available, which was equivalent to an inorganic fertilization after 12 wk incubation. This would indicate that seaweed can enhance the fertilizing effect of pellets, as can be verified with the level of N available recorded. The treatments with different pellets and doses but without seaweed generated levels of N available equal to the control treatment C (soil alone) in all the time periods assessed (Table 4). This would be explained by the low levels of total N found in the two basic components of the pellets, which are ash and sludge (Table 3).

The rapid release of N available observed in the treatment with inorganic fertilizer is attributed to urea hydrolysis by the urease enzyme (Kumar et al., 2007), whereas mineralization of organic amendments is required to release N available soil. The effect of the treatments with addition of seaweed was similar to that found by Rothlisberger-Lewis et al. (2016), who reported that adding lipids extracted from the microalgae *Nannochloropsis salina* D.J. Hibberd in a loam-clay-sandy soil resulted in a 36% increase of N mineralized compared to the control treatment after a time period of one year. In another study, Hseu and Huang (2005) reported that adding wastewater biosolids to three soil types increased the concentration of mineral N, with a lower concentration of N from the second week, which was attributed to a decreased microbial activity over time or exhaustion of readily mineralizable N. With respect to the amendments with pellets alone, the results were similar to those found by Ríos et al. (2012). They amended an Alfisol with paper mill sludge and reported an amount of 12 mg kg^{-1} N available after 15 d incubation, which is considered a low level according to reference levels (Vidal, 2007).

The increased net N mineralization in treatments with seaweed can be attributed to the contributions of carbohydrates and N content of the seaweed since *Ulvaceae* have a phycocolloid, which is a carbohydrate of high molecular weight called 'ulvan' (Lahaye, 2001). This phycocolloid is composed of rhamnose, xylose, glucuronic acid and iduronic acid with a high presence of sulfate groups (Paradossi et al., 2002), resulting in an increase in microorganisms that mineralize N in the soil. A greater release of inorganic N in treatment $AL_{10}PE3_{10}$, can be explained by the contribution of the seaweed and a higher C content due to a higher content of sludge in pellet 3 (20%). A higher level of OM provides a good supply of food for soil microorganisms, which release nutrients through its decomposition.

Table 4. Net N mineralization N (MNt) for each time period in the incubation experiment of pelletized waste materials alone and combined with seaweed in an Entisol.

Treatments	2 wk	4 wk	6 wk	8 wk	12 wk
	MNt				
	mg kg^{-1}				
T0	3.06c	3.68c	7.49c	17.08c	8.31c
TF	91.81a	97.89a	98.49a	114.72a	73.53a
AL_{10}	42.91b	51.60b	50.16b	58.06b	47.25b
$PE1_{10}$	3.04c	5.09c	6.95c	15.41c	7.18c
$PE2_{10}$	4.53c	6.62c	8.99c	16.51c	9.67c
$PE3_{10}$	4.52c	4.92c	8.19c	19.85c	9.28c
$AL_{10}PE1_{10}$	37.79b	40.08b	49.76b	48.27b	43.79b
$AL_{10}PE2_{10}$	33.19b	43.09b	47.12b	53.35b	44.06b
$AL_{10}PE3_{10}$	35.04b	44.79b	46.87b	61.11b	55.14ab
CV %	17.41	18.73	9.58	17.81	27.51
MSD	14.16	17.73	9.87	22.89	26.08

T0: Unamended soil; TF: soil plus inorganic fertilization; AL_{10}: soil + 10 t ha^{-1} seaweed; $PE1_{10}$: 10 t ha^{-1} pellet 1; $PE2_{10}$: 10 t ha^{-1} pellet 2; $PE3_{10}$: 10 t ha^{-1} pellet 3; $AL_{10}PE1_{10}$: 10 t ha^{-1} pellet 1 + 10 t ha^{-1} seaweed; $AL_{10}PE2_{10}$: 10 t ha^{-1} pellet 2 + 10 t ha^{-1} seaweed; $AL_{10}PE3_{10}$: 10 t ha^{-1} pellet 3 + 10 t ha^{-1} seaweed; CV: coefficient of variation; MSD: minimum significant difference.
Different letters in the columns indicate significant differences ($p \leq 0.05$).

N mineralization potential

Results of N mineralization potential (MP) were obtained from the MNt incubation trial and produced first-order nonlinear equations for each treatment (Table 5). The release dynamics of N (Figure 1) shows that the mineralization rate constants (k) range between 0.19 and 1.57 so that a lower k in the treatments indicates a slower N-release rate over time (Ruiz et al., 2008). The treatments with pellets and seaweed combined had higher values of N mineralization potential compared to the treatments only with pellets. Treatment $AL_{10}PE3_{10}$, which included seaweed and pellets, had the highest potentially mineralizable N value (*No*) that reached a higher level only in the TF treatment. In fact, the TF treatment recorded the highest *No* and k values, which indicates a faster N release rate over time.

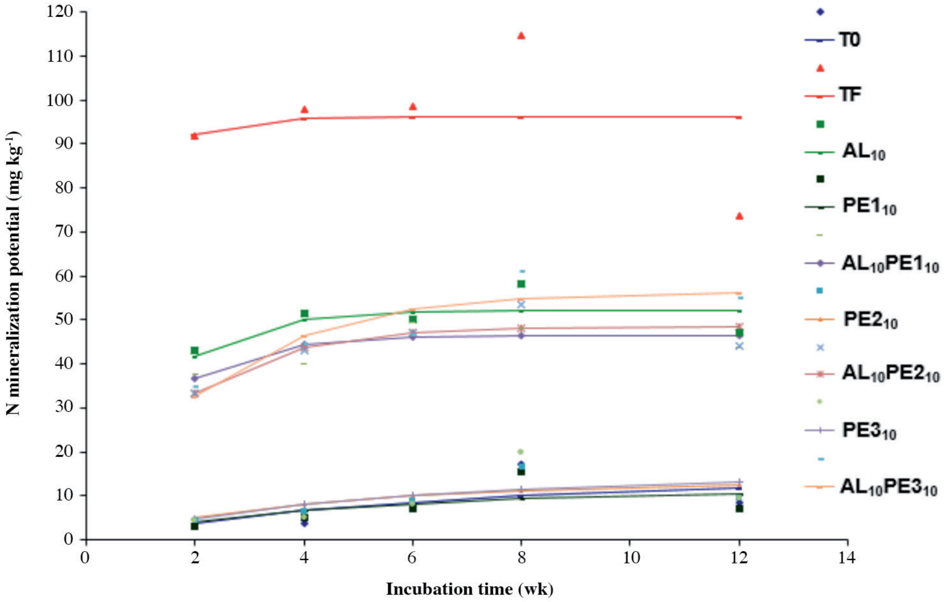
The values found for k agree with those obtained by Hernández et al. (2002), who reported values between 0.22 and 1.14 in clay soils and sandy soils amended with biosolids.

Table 5. Potentially mineralizable N (*No*), mineralization rate constant (k), and first-order adjustment equations in the incubation experiment of pelletized waste materials alone and combined with seaweed in an Entisol.

Treatments	*No*	k	Equation
T0	13.46 ± 5.83	0.168 ± 0.14	$N_{mt} = 13.46\ (1-e^{(-0.168\ t)})$
TF	96.16 ± 5.68	1.570 ± 1.50	$N_{mt} = 96.16\ (1-e^{(-1.570\ t)})$
AL_{10}	52.29 ± 1.90	0.806 ± 0.20	$N_{mt} = 52.29\ (1-e^{(-0.806\ t)})$
$PE1_{10}$	11.14 ± 3.17	0.222 ± 0.14	$N_{mt} = 11.14\ (1-e^{(-0.222\ t)})$
$AL_{10}PE1_{10}$	46.39 ± 2.17	0.776 ± 0.21	$N_{mt} = 46.39\ (1-e^{(-0.776\ t)})$
$PE2_{10}$	13.19 ± 3.52	0.238 ± 0.15	$N_{mt} = 13.19\ (1-e^{(-0.238\ t)})$
$AL_{10}PE2_{10}$	48.59 ± 2.63	0.578 ± 0.14	$N_{mt} = 48.59\ (1-e^{(-0.578\ t)})$
$PE3_{10}$	14.54 ± 5.10	0.197 ± 0.15	$N_{mt} = 14.54\ (1-e^{(-0.197\ t)})$
$AL_{10}PE3_{10}$	56.66 ± 3.41	0.427 ± 0.09	$N_{mt} = 56.66\ (1-e^{(-0.427\ t)})$

N_{mt}: Mineral N accumulated during a specific time period (mg kg^{-1}); t: time period (wk); T0: unamended soil; TF: soil plus inorganic fertilization; AL_{10}: soil + 10 t ha^{-1} seaweed; $PE1_{10}$: 10 t ha^{-1} pellet 1; $PE2_{10}$: 10 t ha^{-1} pellet 2; $PE3_{10}$: 10 t ha^{-1} pellet 3; $AL_{10}PE1_{10}$: 10 t ha^{-1} pellet 1 + 10 t ha^{-1} seaweed; $AL_{10}PE2_{10}$: 10 t ha^{-1} pellet 2 + 10 t ha^{-1} seaweed; $AL_{10}PE3_{10}$: 10 t ha^{-1} pellet 3 + 10 t ha^{-1} seaweed.

Figure 1. N mineralization potential calculated based on the net N mineralization in the incubation experiment of pelletized waste materials alone and combined with seaweed in an Entisol over time.



T0: Unamended soil; TF: soil plus inorganic fertilization; AL_{10}: soil + 10 t ha^{-1} seaweed; $PE1_{10}$: 10 t ha^{-1} pellet 1; $PE2_{10}$: 10 t ha^{-1} pellet 2; $PE3_{10}$: 10 t ha^{-1} pellet 3; $AL_{10}PE1_{10}$: 10 t ha^{-1} pellet 1 + 10 t ha^{-1} seaweed; $AL_{10}PE2_{10}$: 10 t ha^{-1} pellet 2 + 10 t ha^{-1} seaweed; $AL_{10}PE3_{10}$: 10 t ha^{-1} pellet 3 + 10 t ha^{-1} seaweed.

The *k* values obtained in this study were higher than those found by Hirzel et al. (2010), who reported values between 0.075 and 0.10 by adding broiler litter to an Andisols. Hseu and Huang (2005) found that sandy soils amended with different doses of biosolids showed low values of *No* due to low moisture retention and soil biological activity.

Production parameters in lettuce with the use of pellets and seaweed in pots

The ANOVA results indicate that the aerial biomass (AB), relative growth rate (RGR), total N (Nt) and level of chlorophyll (CL) evaluated in lettuce were different ($p \leq 0.05$) when pellets were combined with seaweed compared to those treatments without seaweed (Table 6). Higher values for RGR, Nt and CL ($p \leq 0.05$) were observed when seaweed was added with dose/pellet type (Table 7). There was an interaction between factors (seaweed and dose/pellet type) only for AB (Table 6), with a more positive interaction for the combination of pellets with seaweed than for pellets without seaweed (Table 8). The mean value for AB in all the treatments consisting of pellets+seaweed was 7-fold higher than the value recorded for the treatments without seaweed and those with only pellets. The values obtained in the combined pellet+seaweed treatments were significantly equal to those of AL_{10}. The combined treatment $AL_{10}PE1_{20}$ reached 4.16 g pot^{-1} AB in lettuce (Table 8), which can be attributed to the composition of pellet 1 since its higher amount of ash would result in a greater contribution of minerals to the soil, such as Ca and K (Table 3).

For the treatments consisting of only types of pellets 1, 2 and 3 in different doses, no differences were found for RGR, Nt and CL with respect to T0 (Table 8). This means that the types of pellets and doses evaluated did not increase the RGR, Nt and CL in the Entisol used in this study, which shows low nutrient availability and MO content (Table 3). The stimulating effect that *U. lactuca* has on aerial biomass is due to the macro and micronutrients present in the composition of seaweed (Table 2), and also to the presence of hormones such as auxin, gibberellins and cytokinins (Pramanick et al., 2013). In addition, the nutritional value and OM content of the pellets (Table 2) increases these elements in the soil, helping improve the physical properties of the soil (Gallardo et al., 2012).

When considering the results of RGR separately with or without seaweed, it can be indicated that treatments

Table 6. F values of ANOVA for aerial biomass (AB), relative growth rate (RGR), total N (Nt), and content of chlorophyll (CL) of the experiment of lettuce in pots, amended with pelletized waste materials with and without seaweed added.

	F Values			
Source of variation	AB	RGR	Nt	CL
Seaweed (A)	1298.36**	175.82**	12.96*	58.50**
Dose/pellet type (B)	2.01ns	3.71*	0.67ns	3.30*
A × B	2.52*	0.81ns	0.79ns	1.28ns

*, **Significant at the 0.05 and 0.01 probability levels, respectively; ns: nonsignificant.

Table 7. Effect of the addition of seaweed on the relative growth rate (RGR), total N (Nt) and content of chlorophyll (CL) of lettuce plants grown in pots, amended with pelletized waste materials with and without seaweed added.

	RGR	Nt	CL
	g g^{-1} d^{-1}	%	SPAD
With seaweed	0.061a	0.97a	27.54a
Without seaweed	0.037b	0.87b	23.55b
CV, %	14.04	11.80	7.90

CV: Coefficient of variation. Different letters in the columns indicate different values according to Tukey test ($p \leq 0.05$).

Table 8. Aerial biomass (AB), relative growth rate (RGR), total N (Nt), and chlorophyll (CL) content for amendments consisting of pelletized waste materials from the paper industry with and without seaweed added.

	AB	RGR	Nt	CL		AB	RGR	Nt	CL
Treatment	Without seaweed				Treatment	With seaweed			
	g pot^{-1}	g g^{-1} d^{-1}	%	SPAD		g pot^{-1}	g g^{-1} d^{-1}	%	SPAD
T0	0.42C	0.040	0.83	22.53	AL_{10}	4.67A	0.066ab	0.96	28.03ab
$PE1_{10}$	0.69C	0.044	0.93	25.40	$AL_{10}PE1_{10}$	3.37B	0.063abc	1.04	28.10ab
$PE1_{20}$	0.68C	0.044	0.95	24.50	$AL_{10}PE1_{20}$	4.16AB	0.069a	1.03	26.20bc
$PE1_{30}$	0.65C	0.027	0.87	22.90	$AL_{10}PE1_{30}$	3.40B	0.058c	0.96	23.17c
$PE2_{10}$	0.46C	0.032	0.78	21.43	$AL_{10}PE2_{10}$	3.74AB	0.056c	0.98	25.67bc
$PE2_{20}$	0.44C	0.043	0.83	22.77	$AL_{10}PE2_{20}$	3.49B	0.060bc	1.03	28.10ab
$PE2_{30}$	0.46C	0.039	0.89	26.13	$AL_{10}PE2_{30}$	4.02AB	0.059bc	0.93	30.57a
$PE3_{10}$	0.44C	0.024	0.82	23.57	$AL_{10}PE3_{10}$	3.50B	0.056c	0.97	27.97ab
$PE3_{20}$	0.49C	0.035	0.89	23.50	$AL_{10}PE3_{20}$	3.57B	0.057c	0.94	28.63ab
$PE3_{30}$	0.60C	0.040	0.91	22.77	$AL_{10}PE3_{30}$	4.01AB	0.063abc	0.87	28.93ab
CV%	19.19	22.41	9.19	8.70	CV %	13.04	7.50	14.40	7.53
MSD	0.30	0.024	0.23	6.00	MSD	0.84	0.007	0.24	3.55

T0: Unamended soil; $PE1_{10}$: 10 t ha^{-1} pellet 1; $PE1_{20}$: 20 t ha^{-1} pellet 1; $PE1_{30}$: 30 t ha^{-1} pellet 1; $PE2_{10}$: 10 t ha^{-1} pellet 2; $PE2_{20}$: 20 t ha^{-1} pellet 2; $PE2_{30}$: 30 t ha^{-1} pellet 2; $PE3_{10}$: 10 t ha^{-1} pellet 3; $PE3_{20}$: 20 t ha^{-1} pellet 3; $PE3_{30}$: 30 t ha^{-1} pellet 3; AL_{10}: soil + 10 t ha^{-1} seaweed; $AL_{10}PE1_{10}$: 10 t ha^{-1} pellet 1 + 10 t ha^{-1} seaweed; $AL_{10}PE1_{20}$: 20 t ha^{-1} pellet 1 + 10 t ha^{-1} seaweed; $AL_{10}PE1_{30}$: 30 t ha^{-1} pellet 1 + 10 t ha^{-1} seaweed; $AL_{10}PE2_{10}$: 10 t ha^{-1} pellet 2 + 10 t ha^{-1} seaweed; $AL_{10}PE2_{20}$: 20 t ha^{-1} pellet 2 + 10 t ha^{-1} seaweed; $AL_{10}PE2_{30}$: 30 t ha^{-1} pellet 2 + 10 t ha^{-1} seaweed; $AL_{10}PE3_{10}$: 10 t ha^{-1} pellet 3 + 10 t ha^{-1} seaweed; $AL_{10}PE3_{20}$: 20 t ha^{-1} pellet 3 + 10 t ha^{-1} seaweed; $AL_{10}PE3_{30}$: 30 t ha^{-1} pellet 3 + 10 t ha^{-1} seaweed; CV: coefficient of variation; MSD: minimum significant difference.

Different letters in the columns indicate differences according to Tukey ($p \leq 0.05$). Different capital letters in the rows for AB indicate differences according to Tukey test ($P \leq 0.05$).

with seaweed produced RGR values that were on average 60% higher than those recorded without seaweed (Table 8). Treatment $AL_{10}PE1_{20}$ had a higher RGR in lettuce ($p \leq 0.05$), with respect to treatments $AL_{10}PE1_{30}$, $AL_{10}PE2_{10}$, $AL_{10}PE2_{20}$, $AL_{10}PE2_{30}$, $AL_{10}PE3_{10}$, $AL_{10}PE3_{20}$, indicating that 20 t ha^{-1} of Pellet 1 with seaweed has a positive effect on the RGR of lettuce. This resulted in a higher daily production rate of biomass, although no differences were found when compared to treatment AL_{10} with only seaweed. This indicates that seaweed under study can improve pellets made of waste materials from the paper industry by providing nutrients to low fertility soils as shown in the positive growth response observed in the lettuce plants, which confirms the high potential of this mixture as a fertilizer. Furthermore, no differences were found ($p > 0.05$) in RGR values in lettuce for the treatments with different pellets and doses. These results are consistent with those obtained by Hernández-Herrera et al. (2014), who reported an increased growth in lettuce plants using liquid extracts of *U. lactuca* and *Padina gymnospora* (Kützing) Sonder.

No differences were found between the combined pellets+seaweed amendments in terms of Nt content in the lettuce leaves. Similar results were obtained for treatments consisting of only pellets. Instead, a higher concentration of Nt was observed in the lettuce leaves with the combined pellets+seaweed treatments, with values 11% higher than the those that included only pellets. Regarding the CL content in the combined pellets+seaweed treatments, treatment $AL_{10}PE2_{30}$ recorded the highest value ($p \leq 0.05$) compared to the levels observed in $AL_{10}PE1_{20}$, $AL_{10}PE1_{30}$ and $AL_{10}PE2_{10}$, although it was not different from that of AL_{10}. Thus, Pellet 2 applied to the soil at 30 t ha^{-1} plus seaweed at 10 t ha^{-1} increased chlorophyll level in lettuce leaves. In general, the amendments that combined the use of pellets and seaweed produced a 17% increase in CL content compared to the treatments that include only pellets. This indicates that the seaweed studied has a positive effect on the level of chlorophyll in leaves lettuce. On the contrary, no differences were found between the treatments consisting only of pellet type and dose. The effect of seaweed on CL content found in this study is consistent with results reported by Selvam and Sivakumar (2013). These authors found that when spraying *Vigna mungo* (L.) Hepper plants with extract of *Ulva reticulata* Forsskal seaweed at 2%, the levels of chlorophyll, proteins, sugars and starch increased significantly.

The increase in chlorophyll content can be explained by the bioactive components found in *U. lactuca* extracts, such as ascorbic acid, betaine, glutathione and proline, which would actively participate in the alleviation during stress periods of plants (Ibrahim et al., 2014). A study conducted by Mansori et al. (2015) showed that plants of sage (*Salvia officinalis* L.) under water stress conditions showed increased glycine betaine levels, but these decreased significantly when extracts of *Ulva rigida* C. Agardh at 50% were applied. Glycine betaine is abundant mainly in chloroplasts where it participates in the adjustment and protection of the thylakoid membrane, and also helps stabilize the quaternary structures of complex proteins in the photosystem II, thereby maintaining photosynthetic efficiency (Genard et al., 1991; Papageorgiou and Murata, 1995). In general, the significant increase in the production parameters of lettuce as the result of the combined application of seaweed and pellets indicates that seaweed exerts a positive effect on pellets by enhancing their performance as soil amendments.

CONCLUSIONS

The treatments with the combined use of pellets and seaweed stimulated N mineralization in soil during all the incubation time. The different pellets and doses applied without seaweed added did not increase N mineralization in the soil.

The combined pellets + seaweed treatments had a higher N mineralization potential compared to treatments without seaweed application. These also produced higher biomass gains and growth in lettuce, as well as higher chlorophyll levels and total N in leaves. On the contrary, treatments of pellets without seaweed did not affect these parameters.

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