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# Decomposition of both *Eragrostis plana* biomass and soil influences the phytotoxicity and chemical characteristics of extracts

# Decomposição de biomassa de *Eragrostis plana* e solo influenciam a fitotoxidade e as características químicas dos extratos

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**Abstract**

*Eragrostis plana* is an exotic and resilient weed in southern South America rangeland and pastureland. The goal of this study was to determine the phytotoxicity and chemical characteristics of extracts obtained from *E. plana* shoot residue. Bioassays evaluating the phytotoxicity of the extracts on the development of *Triticum aestivum* seedlings were completely randomized and consisted of four replicates in a two-factorial scheme. The levels of factor A consisted of shoot biomass, biomass + soil, and soil material, whereas the levels of factor B consisted of the incubation periods of 1, 3, 7, 10, 14, and 21 days. The radicle length of *T. aestivum* seedlings was reduced by biomass and biomass + soil after 1 and 10 days and after 1, 3, 10, and 14 days of incubation, respectively. The hypocotyl length was reduced by the biomass and biomass + soil extracts after 10 and 14 days and after 1, 3, 10, 14, and 21 days of incubation, respectively. The soil extracts allowed longer *T. aestivum* radicles and hypocotyls than did the control. The pH values did not differ between biomass, biomass + soil, and soil, whereas the highest electrical conductivity values were registered for the biomass extracts, followed by the biomass + soil and soil extracts, with the last showing extremely low levels. The total phenolic concentrations in all decomposed materials were higher during the first few days but gradually decreased with increasing incubation period. The combination of biomass and soil increased the phytotoxicity of the biomass, necessitating additional studies on the interaction between the allelochemicals produced by these plants and the soil.

**Additional keywords:** allelopathy; inhibition; South African lovegrass; total phenolics.

**Resumo**

*Eragrostis plana* é uma planta invasora exótica e resiliente nos campos e pastagens do sul da América do Sul. O objetivo deste estudo foi determinar a fitotoxidade e as características químicas de extratos obtidos de resíduos da parte aérea de *E. plana*. Bioensaios avaliando a fitotoxidade dos extratos no desenvolvimento de plântulas de trigo foram conduzidos em delineamento inteiramente casualizado, com quatro repetições, em esquema bifatorial. Os níveis do fator A foram os materiais biomassa de parte aérea, biomassa + solo e solo, enquanto os níveis do fator B consistiram nos períodos de incubação de 1; 3; 7; 10; 14 e 21 dias. O comprimento de radícula de trigo foi reduzido pela biomassa e biomassa + solo, após 1 e 10 dias, e após 1; 3; 10 e 14 dias de incubação, respectivamente. O comprimento de hipocótilo foi reduzido pelos extratos da biomassa e biomassa + solo, após 10 e 14 dias, e após 1; 3; 10; 14 e 21 dias de incubação, respectivamente. Os extratos de solo permitiram radículas e hipocótilos maiores que a testemunha. Os valores de pH não diferiram entre biomassa, biomassa + solo e solo, enquanto os maiores valores de condutividade elétrica foram registrados para o extrato de biomassa, seguido pelo de biomassa + solo e finalmente solo, com o último apresentando níveis extremamente baixos. A concentração de fenóis totais em todos os materiais decompostos foi maior nos primeiros dias, reduzindo-se gradualmente com o aumento do período de incubação. A combinação de biomassa e solo aumentou a fitotoxidade da biomassa, indicando a necessidade de estudos adicionais sobre a interação entre os aleloquímicos produzidos por essas plantas e o solo.

**Palavras-chave adicionais:** alelopatia; capim-annoni-2; fenóis totais; inibição.

## Introduction

Decomposition of plant tissue is the most common phenomenon associated with the release of allelochemicals, such as phenols (Wojcik-Wojtkowiak et al., 1990). Phenolic compounds can play a significant role in ecological relations between plant populations and can inhibit the presence of susceptible species. This inhibition can occur even when phenolic compounds are in low concentrations due to additive and synergistic effects that occur between chemical compounds in the soil solution (Blum, 1996, Reigosa et al., 1999).

Previous studies have indicated the need for the oxidation of phenolic compounds in order to express biological activity. Consequently, the phytotoxicity of these compounds can vary based on the activity of enzymes and oxidants present in the environment, even when the concentrations and type of phenolic compounds are constant. Oxidation activation may explain much of the observed variation between individuals and ecosystems (Appel, 1993).

Allelopathic activity has been attributed to *Eragrostis plana*, a resilient invasive weed of South American rangeland and pastureland that has already infested more than a million hectares. In a pioneering study, Coelho (1986) identified a deleterious effect on the soil of an area invaded by *E. plana* with respect to the germination and seedling development of *Trifolium repens* L. and *Lolium multiflorum* Lam. Ferulic, caffeic, vanillic and *p*-coumaric acids have been identified in the leaf and root extracts of *E. plana*, and catechin and epicatechin have been identified in the leaf extracts (Favaretto et al., 2015). Triacylglycerol and rotenoid were also identified in the fractions of the acetate extracts (Klein, 2015).

The biomass of *E. plana* also reduces the germination of *Paspalum notatum* Flugge and *Setaria sphacelata* (Schum.) Stapf & C.E.Hubb.) (Ferreira et al., 2008). In addition, leaf and root extracts impair the development of *T. repens* seedlings (Favaretto et al., 2011). *Ipomoea grandifolia* (Dammer) O'Donell and *Euphorbia heterophylla* L. germination and seedling development are also reduced by the shoot and root extracts of *E. plana* (Dalbosco, 2013, Silva, 2014).

In this context, the main objectives of this study were to examine the effects of the decomposition time of *E. plana* Nees shoots and soil on the phytotoxicity of its aqueous extracts and determine the chemical characteristics of the extracts.

## Material and methods

### Biomass preparation and incubation

The shoot biomass of 200 *E. plana* plants was randomly collected in an infested pastureland in the municipality of Abelardo Luz (SC) (coordinates: -26.527820, -52.255808) in April 2015 at the end of their reproductive cycle. The plants were manually cut 1 cm above the soil surface and removed, and the plant samples were kept in a freezer at -26 ± 2 °C until drying. The sample was dried in a forced-air circulation oven at 40 °C for 72 hours. Shortly after drying, the samples were milled using a Wiley type mill (2-mm screen).

The soil used in the experiment was obtained from a *Urochloa brizantha* (A.Rich) R.D.Webster pasture area that was not contaminated with *E. plana* (coordinates: -25.444292, -52.445915) in the experimental field of the Federal University of Fronteira Sul in the municipality of Laranjeiras do Sul (PR) in May 2015. Approximately 1 kg of dystroferric Red Latosol of a 10-cm-deep rectangle was sampled using a shovel. The soil collected was oven-dried in a forced-air circulation oven at 40 °C for 72 hours. After drying, the soil was ground using a ceramic mortar and pestle and passed through a sieve (2 mm).

To simulate the degradation of biomass, biomass + soil, and soil, 500-mL glass jars were used, and three holes (2 mm) were drilled through the lids to allow gas exchange. The biomass treatment received 25 g of dried *E. plana* shoots and 75 mL of distilled water. The soil + biomass treatment received 25 g of dried shoots, 25 g of soil, and 82.5 mL of distilled water. The soil treatment received 25 g of soil and 7.5 mL of distilled water. The amount of water was calculated to allow the soil to reach field capacity. After they were assembled, the jars were incubated in a dark room at a controlled temperature (18 °C) for 1, 3, 7, 10, 14, or 21 days. The water was replaced daily according to the mass lost due to evaporation.

### Preparation of aqueous extracts

Volumes of 340, 350, and 290 mL of distilled water were added to the biomass, biomass + soil, and soil samples, respectively, to obtain aqueous extracts. The water was added directly to the glass jar in which the material was incubated. The jars were closed and then shaken by an orbital shaker incubator at 200 rpm for 10 minutes at 25 °C.

Afterward, the material sat on the bench for 5 minutes before being filtered through a glass funnel filled with gauze. The liquid portion was centrifuged at 3,900 rpm for 40 minutes at 25 °C. The supernatant was filtered using filter paper (25-µm pores), and the amount of solution recovered was approximately 270 mL for all treatments. Each extract was fractionated into three portions: one for the bioassay; one for the determination of total phenols, pH and electrical conductivity; and one for chromatographic analysis. The extracts obtained from the incubated materials were stored under refrigeration for 1 day, after which they sat for 3 hours at room temperature prior to use in phytotoxicity bioassays.

### Bioassay for the phytotoxicity of aqueous extracts

The bioassay to test the phytotoxicity of the extracts was organized as a completely randomized design with four replicates. The experimental units

consisted of gerbox-type boxes lined with two layers of blotting paper; each box received 3.7 mL of aqueous extract or distilled water (control) and 20 *Triticum aestivum* cv. IPR Catuara seedlings.

To obtain seedlings, approximately 5,000 seeds were germinated in plastic trays lined with blotting paper that was moistened with distilled water at a ratio of 1:2.5 (w/w). The trays were wrapped in aluminum foil to avoid exposing the seedlings to light and incubated in a growth chamber at 20 °C (Brasil, 2009). The blotting paper was kept moist during the incubation period by replenishing the distilled water lost by evaporation. The *T. aestivum* seedlings reached the root length for bioassay implementation 72 hours after sowing. Classification was performed by separating 1,440 seedlings that had radicle lengths between 5 and 7 mm. These seedlings were arranged in gerboxes that were wrapped in aluminum foil and incubated in growth chambers at 20 °C for 48 hours. The entire process of obtaining and manipulating the seedlings was performed in a room using green light only to avoid activating phytochrome and, consequently, seedling shedding.

After 48 hours of incubation, the radicle and hypocotyl lengths of the seedlings were measured using a digital caliper. The mean radicle and hypocotyl lengths from the 20 seedlings of each replicate were utilized for statistical analysis.

**pH, electrical conductivity, and total phenol content of aqueous extracts**

The pH and electrical conductivity of the extracts were measured at room temperature (~25 °C) using a pH meter and a conductivity meter, respectively.

To determine the total phenol content, sample preparation and extraction procedures were performed. Seventy-five milliliters of acetone and 25 mL of extract were mixed together and then stirred for 12 hours using a magnetic stirrer. The mixed solution was subsequently filtered through filter paper (25-µm pores) in a vacuum to separate proteins and lipids, after which the supernatant was discarded. The acetone was then removed using a rotary evaporator at 40 °C. The clear extract was then fractionated three times with 50 mL of hexane, after which the hexane fraction was discarded. The remaining solution was fractionated three times with 50 mL of diethyl ether. The ethereal solution was then placed on a rotary evaporator at 40 °C for ether evaporation, after which the ethereal solution was dried with anhydrous $Na_2SO_4$. The obtained extracts were then weighed.

The quantification of total phenols was performed in accordance with the methodology proposed by López & Juan (2013). The composition of the cuvettes for reading in the spectrophotometer consisted of 100 µL of extract, 600 µL of $Na_2CO_3$ (7.5%), 700 µL of distilled water, and 200 µL of Folin–Ciocalteau reagent. This mixture was heated to 50 °C for 10 minutes and diluted with 1.5 mL of distilled water before reading. The calibration curve of the spectrophotometer was constructred using solutions of 0, 10, 20, 30, and 40 µg $mL^{-1}$ of gallic acid. The phenolic substances present in the evaluated samples were reported in micrograms of gallic acid equivalents per milliliter of aqueous extract.

**Chromatographic profiles of the aqueous extracts**

To analyze the behavior of the chemical compounds during incubation, samples from the ethereal extracts described in the previous section were used. A 200-µL volume of ethereal extract from each sample was mixed together with 1.8 µL of spectroscopic-grade methyl alcohol. The samples were then filtered through a 0.45-µm-pore membrane filter for subsequent 10-µL-volume injections of each sample into a Varian® model 920-R6 liquid chromatograph equipped with an ACE® C18 column (100 × 2, 1 mm, 1.8 µm) in accordance with the methodology proposed by Silva (2016). A mobile phase gradient composed of two solvent mixtures was used: A) water and acetic acid (98:2) and B) water, acetic acid, and acetonitrile (58:2:40). The system was maintained at 30 °C and had a flow rate of 2.0 mL $min^{-1}$.

**Statistical analysis**

The data were subjected to analysis of variance (ANOVA) ($p < 0.05$) and subsequent regression analysis or multiple comparison tests of means using Genes software (Cruz, 2013). When applicable, the simplest models capable of adjusting to the observed data were adopted to allow a prediction that resulted in the smallest square sum value of the residual. When it was not possible to adjust the means to regression models, lines linked the means.

## Results and discussion

The results of the factorial ANOVA for the radicle and hypocotyl length variables showed a significant ($p < 0.01$) interaction between the material (biomass, biomass + soil, and soil) and incubation period (1, 3, 7, 10, 14, and 21 days) factors.

**Effects of aqueous extracts on *Triticum aestivum* radicle length**

Regression analyses were performed between the degradation period and *T. aestivum* radicle length for each incubated material. No models were identified that adequately represented the biological responses to treatment with biomass (Figure 1A) or biomass + soil (Figure 1B); thus, those data were combined. A second-order polynomial model ($Y = a + bX + cX^2$) fit the data between the soil incubation period and *T. aestivum* radicle length (Figure 1C).

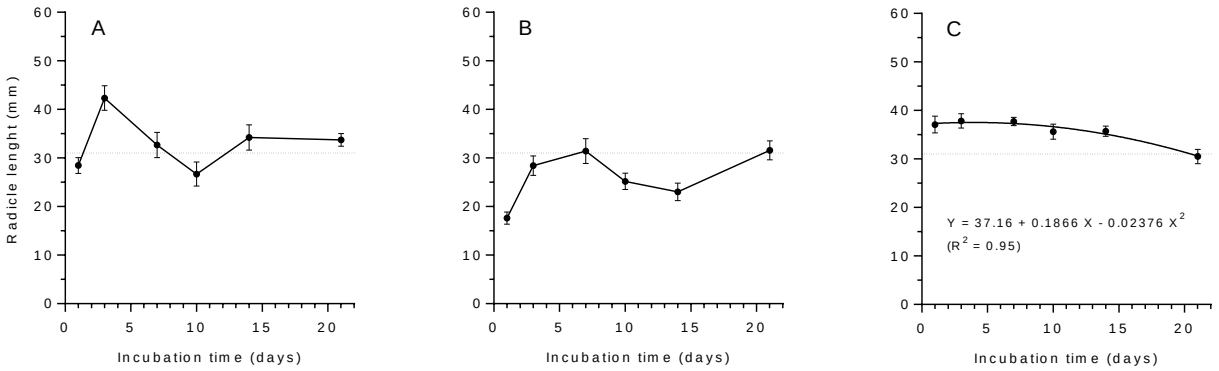


**Figure 1 -** Length of *Triticum aestivum* seedlings radicle in treatments with aqueous extract of *Eragrostis plana* biomass (A), biomass + soil (B) and soil (C) in different periods of incubation (days) in relation to the control with distilled water. The dots represent the means of the treatments and the standard error the variability between the replicates. The curve in (C) represents a second-order polynomial model; Where Y is the radicle length at a given time (days) of material incubation X.

The aqueous extracts of the biomass and biomass + soil treatments reduced the radicle length below that of the control. The soil aqueous extracts positively affected the *T. aestivum* radicle length for the majority of incubation times, and these radicle lengths approached those of the control only after 21 days of incubation.

Reduction of *Arabidopsis thaliana* (L.) Heynh. seedling root length has been attributed to catechin (Duke et al., 2009). Also, despite being attributed to the antioxidant properties of catechin when tested alone, the results of Sugihara et al. (2001) and Kumamoto et al. (2001) suggest that the presence of ions such as iron ($Fe^{2+}$) modifies these characteristics and provides pro-oxidative activities. The Red Latosol of Paraná presents high concentrations of iron, due to the chemical features of the material of basaltic origin (Melo et al., 2008). Therefore, it is hypothesized that the iron present in the sampled soil (91 mg $dm^{-3}$) may have increased the phytotoxicity of the biomass + soil extracts.

The aqueous biomass extracts increased the radicle size of the *T. aestivum* seedlings in comparison to that of the control seedlings at four incubation periods (3, 7, 14, and 21 days). On the first and 10$^{th}$ days of incubation, however, these effects were negative and reduced the radicle length by approximately 10% compared with that of the control.

The biomass + soil extract resulted in *T. aestivum* seedlings that had radicle lengths similar to those observed in the control only on the seventh and 21$^{st}$ days of incubation. In the other incubation periods, however, the biomass + soil aqueous extract reduced the length of the radicle in relation to that of the control, reaching 40% by the first day of incubation.

The soil aqueous extracts positively affected the development of *T. aestivum* radicles, as the values were higher than those of the control during the majority of the incubation period. From the first to the seventh day of incubation, the radicle length was approximately 20% greater, and between the 10$^{th}$ and the 14$^{th}$ day of incubation, the radicle length decreased but remained 15% higher than that of the control, reaching similar lengths after the 21$^{st}$ day.

**Effects of aqueous extracts on *Triticum aestivum* hypocotyl length**

Regression analyses were performed between the levels of the incubation period and each level of material type on *T. aestivum* hypocotyl lengths. Because no models were identified that adequately represented the results of phytotoxicity on hypocotyl length in the treatments of biomass and biomass + soil, the data were combined. A second-order polynomial model ($Y = a + bX + cX^2$) best fit the relationship between the phytotoxicity of the soil extract and incubation period (Figure 2C).

In general, the effects of the treatments on radicle length were similar to those observed for the hypocotyl length of *T. aestivum* seedlings. The biomass and biomass + soil treatments reduced the hypocotyl length in comparison to that of the control, which consisted of only distilled water and soil.

The aqueous extracts of the incubated biomass presented lower hypocotyl length values than did the control on the 10$^{th}$ and 14$^{th}$ days of incubation and reached reductions of approximately 15% compared with the control on the 10$^{th}$ day. However, in the other periods, the biomass treatment presented longer hypocotyls than did the control – up to 60% on the third day of incubation (Figure 2A).

The biomass + soil aqueous extracts resulted in lower hypocotyl length values compared with the control values in the majority of the incubation periods evaluated and were up to 50% lower on the first day of incubation. However, the difference in relation to the control varied between periods and reached values close to those observed in the control on the seventh day of incubation (Figure 2B).

Soil aqueous extracts resulted in longer *T. aestivum* hypocotyls compared with those of the control in the majority of the incubation periods. The hypocotyl was more than 20% longer than that observed in the control on the seventh day of incubation.

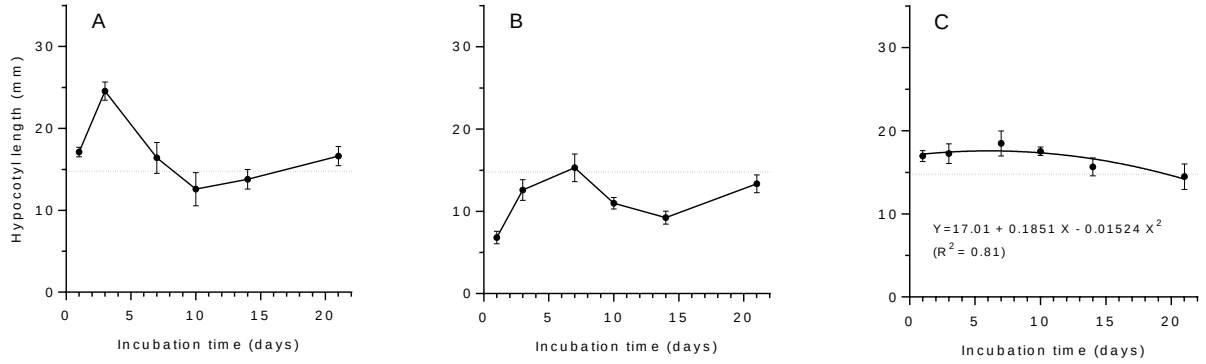


**Figure 2 -** Length of *Triticum aestivum* seedlings hypocotyl in treatments with aqueous extract of *Eragrostis plana* biomass (A), biomass + soil (B) and soil (C) in different periods of incubation (days) in relation to the control with distilled water. The dots represent the means of the treatments and the standard error the variability between the replicates. The curve in (C) represents a second-order polynomial model; Where Y is the radicle length at a given time (days) of material incubation X.

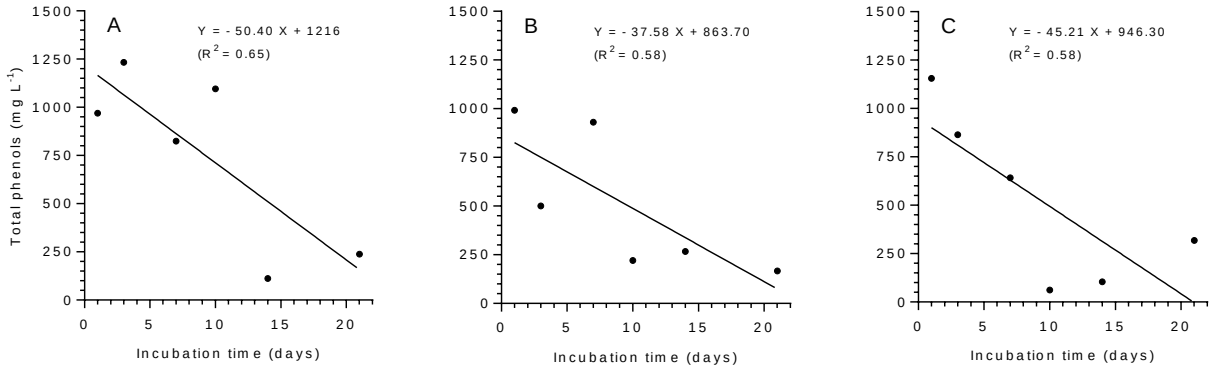


**Figure 3 -** Concentration of total phenols in the aqueous extract of *Eragrostis plana* biomass (A), biomass + soil (B) and soil (C) from the first to the twenty-first day of incubation. The curves represent a first degree polynomial models; Where Y is the concentration of total phenols (mg $L^{-1}$) at a given time (days) of material incubation X.

**pH and electrical conductivity**

The physicochemical properties of a solution can be determining characteristics of the activity, retention in the soil and degradation of phenolic compounds. The attempt to understand and predict the consequences arising from the presence of phenolic compounds in ecological systems requires the measurement of the oxidative capacity of the environment (Appel, 1993).

Among the incubation periods, the pH values of the aqueous extracts of the biomass were all higher than those observed in the biomass + soil and soil extracts (Table 1). The lowest pH value was recorded in the biomass + soil aqueous extract on the first day of incubation (4.6), whereas the highest pH value (7.6) was recorded in the biomass extract after 7 days of incubation.

Although the observed mean pH values differed, there were no significant differences between treatments with respect to the average values of the different incubation periods.

Regarding electrical conductivity, the mean of the different periods of incubation in the biomass treatment was higher than the means of the other treatments. The average values of electrical conductivity in the different incubation periods for the biomass + soil and soil treatments were approximately 28% and 99% lower than those recorded in the biomass treatment, respectively. All means differed according to Tukey's multiple comparison test ($p < 0.05$).

**Table 1 -** Values of pH and electrical conductivity of the aqueous extracts of *Eragrostis plana* shoots biomass, biomass + soil and soil at different incubation periods (days) at room temperature (~ 25 °C).

| Treatments | Incubation period (days) | pH | Electrical conductivity (µS cm$^{-1}$) | pH Average | Electrical conductivity Average |
|---|---|---|---|---|---|
| Biomass | 1 | 5.4 | 1,563 | 6.85 ± 0.32 $^{ns}$ | 1,312.50 ± 72.88 a |
| | 3 | 7.1 | 1,213 | | |
| | 7 | 7.6 | 1,349 | | |
| | 10 | 7.4 | 1,470 | | |
| | 14 | 6.8 | 1,163 | | |
| | 21 | 6.8 | 1,117 | | |
| Biomass + Soil | 1 | 4.6 | 1,313 | 6.31 ± 0.34 $^{ns}$ | 945.33 ± 95.86 b |
| | 3 | 6.6 | 629 | | |
| | 7 | 6.6 | 881 | | |
| | 10 | 6.7 | 954 | | |
| | 14 | 6.6 | 1,082 | | |
| | 21 | 6.6 | 813 | | |
| Soil | 1 | 6.6 | 38 | 6.24 ± 0.10 $^{ns}$ | 18.33 ± 3.88 c |
| | 3 | 6.2 | 14 | | |
| | 7 | 6.4 | 17 | | |
| | 10 | 6.2 | 13 | | |
| | 14 | 6.1 | 15 | | |
| | 21 | 5.9 | 16 | | |

* Treatments with mean ± standard error with different letters in the columns differ by the Tukey test ($p < 0.05$); $^{ns}$ Not significant

Increases in electrical conductivity caused by biomass have been previously reported; an increase in electrical conductivity was observed in the treatment of *Ruta graveolens* L. biomass + soil compared with soil treatment without the presence of the plant residue (Oliva et al., 2002).

The highest values of electrical conductivity were recorded for the biomass treatment, followed by the biomass + soil and soil treatments. The last treatment registered values that differed from those of the first two. Electrical conductivity indicates the presence of ions and salts in a solution and may be associated with the phytotoxicity and osmotic potential of the solution (Abedi-Koupai & Mehdizadeh, 2008). The study of Lawley et al. (2012) on the mechanism involved in the suppression of invasive plants by soil cover with plant residue verified the association between the electrical conductivity of plant extracts and allelopathic potential.

Electrical conductivity increases with increasing concentration of ions or salts in a solution. In the present study, it is hypothesized that the values of electrical conductivity may have increased due to the mineralization of nitrogen present in the *E. plana* biomass or due to the production of organic acids from the degradation of the biomass and its interaction with the soil, among other factors.

**Total phenols**

In all treatments, the total phenolic concentration decreased linearly with increasing incubation time (Figure 3 A-B-C). A comparison of the treatments showed similar concentrations of total phenols both on the first and after the 14$^{th}$ day of incubation. The biomass treatment presented higher total phenolic concentrations until the 10$^{th}$ day of incubation and reached values that were similar to those of the other treatments after the 14$^{th}$ day.

It was possible to estimate the period necessary for the total phenolic concentrations to reach zero by interpolating the regression equations in the different treatments. While the time required for the biomass treatment was approximately 24 days, the time required for the biomass + soil and soil treatments was less, with values of approximately 23 and 21 days, respectively. By adding plant residue that contained high concentrations of phenolic acids to soil that was subsequently incubated for different periods, Martens (2002) reported that ferulic, *trans*-cinnamic, and coumaric acid concentrations and structures remained practically intact for 9 days. However, several factors, such as the activity of phenol oxidase enzymes, may lead to a decrease in the concentration of phenolic compounds (Pind et al., 1994).

Analysis between the treatments allows observing both the positive influence of the biomass and the negative impact of the soil on the concentration of total phenols. It is noteworthy that the soil and biomass + soil extracts presented similar concentrations of total phenols throughout the incubation period. This effect is interesting because the treatment that presented the highest levels of phytotoxicity to *T. aestivum* plants was the biomass + soil treatment. Similarly, the incorporation of *R. graveolens* plant residue into the soil resulted in higher amounts of soluble phenolic compounds compared with the soil alone (Oliva et al., 2002).

No relation was observed between the total phenol concentration and the phytotoxicity of the extracts, which showed independent behavior during the incubation periods. The dynamics regarding the concentration of total phenols, the pH of the solution and electrical conductivity suggest the formation of

phenolates, mainly in the aqueous extracts of the biomass + soil treatment. Phenolates are more stable than phenolic compounds and can also be toxic, depending on the results of the interaction between the phenolic compound and cations, which can give rise to quinones (Appel, 1993), or by altering the biological activity of phenolic compounds (Kumamoto et al., 2001).

When phenolic compounds are ionized and form phenolates, ionic bonds can form, and when the hydrogen is removed from the phenoxyl group, the group can exhibit a negative charge. This reaction is favored under higher pH conditions and occurs when *E. plana* biomass is incorporated into the soil (Table 1). Phenolates are highly reactive because they are already ionized (Appel, 1993). This phenomenon of phenolic compound chelation is hypothetically responsible for the higher toxicity of catechin in the soil compared with that detected in *in vitro* tests (Inderjit et al., 2008).

In addition, soil pH determines the type and stability of the bonds formed between phenolic compounds and organic matter, which directly affects their stability (Castells, 2008). At a pH greater than 8.0, there is a tendency for phenolic compounds to form covalent bonds with organic matter, reducing the availability of the organic matter for absorption by plants; at lower pH values, hydrogen bonds are present, which are characterized by lower strength and higher reversibility (Appel, 1993).

Despite all observations, the absence of correlations between the total phenolic concentration and phytotoxicity negated any conclusions regarding the interaction between the phenolic compounds of the extracts and the development of the radicles and hypocotyls of the *T. aestivum* seedlings. It may be necessary to elucidate the chemical transformations that occur in the extracts obtained from the biomass + soil combination to understand the observed inhibition dynamics. These would allow determining causality by identifying the substances involved in the inhibition of seedling development, which was observed in the combination of biomass + soil.

**Chromatographic profiles of extracts**

The compounds present in the ethereal extract samples could not be properly identified using high-performance liquid chromatography (HPLC), probably due to the low polarity of the diethyl ether used for liquid–liquid extraction. Problems regarding the use of HPLC for the identification and quantification of phenolic compounds extracted for the determination of total phenols by spectrometry are, unfortunately, common (Waterhouse, 2003). Solvents with higher polarity, such as ethyl alcohol or methyl alcohol, might allow better extraction of the phenolic compounds and less extraction of compounds that have low polarity, such as those obtained using diethyl ether, which compromise the separation of the analytes. However, the chromatograms obtained showed that there was degradation of compounds present in the samples.

The ethereal extracts of the degraded biomass underwent the greatest modifications between the first and 10$^{th}$ days of incubation. From day 10, the chromatograms appear very similar to each other; although slight variation between the 10$^{th}$ and 21$^{st}$ days of incubation was observed (Figure 4).

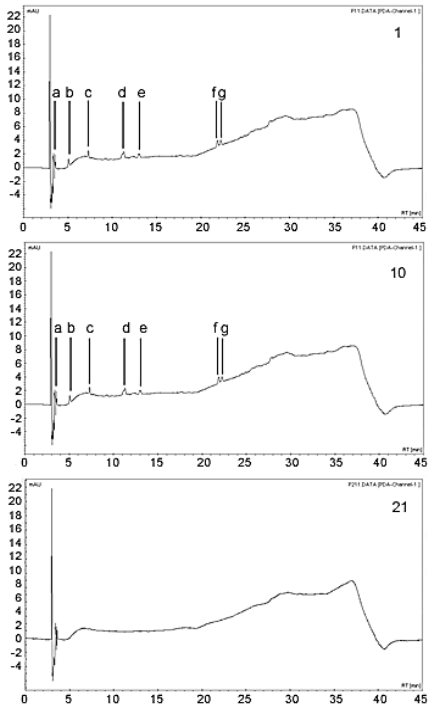


**Figure 4 -** Chromatograms from ethereal extracts of shoots biomass of *Eragrostis plana* incubated for: (1) one day; (10) ten days, and (21) twenty-one days. Chromatograms were obtained using the HPLC technique (High Performance Liquid Chromatography), where Y is the milli-Absorbance Units (mAU) at a given time in minutes (RT) X.

The first and 10th days of incubation exhibited the two lowest *T. aestivum* seedling radicle lengths in the biomass treatment (Figure 1A). These periods coincided with those in which the highest electrical conductivities were recorded. These two degradation periods also coincided with the beginning of the incubation of the compounds observed in the chromatograms and the first subsequent period in which no changes were recorded between the chromatographic profiles. From the 10th day of incubation, the total phenolic concentration of the biomass decreased from values close to 1000 mg $L^{-1}$ to values of 112 and 238 mg $L^{-1}$ on the 14th and 21st days, respectively. From the 10th day, there was also reduced pH variation of the aqueous biomass extract.

The chromatograms of the ethereal extracts of the degraded soil + biomass treatment were altered mainly between the first and third days of incubation (Figure 5). From day 3, the chromatograms were similar to each other, although slight and practically imperceptible changes in the peaks between the 3rd and 21st days of incubation were present.

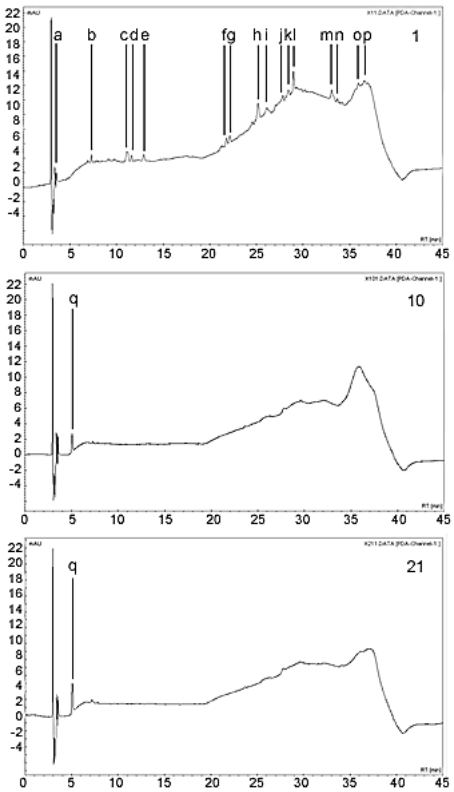


**Figure 5 -** Chromatograms from ethereal extracts of shoots biomass of *Eragrostis plana* + soil incubated for: (1) one day (10) ten days, and; (21) twenty-one days. Chromatograms were obtained using the HPLC technique (High Performance Liquid Chromatography), where Y is the milli-Absorbance Units (mAU) at a given time in minutes (RT) X.

The first day of incubation of the biomass + soil treatment was responsible for the lowest radicle and hypocotyl lengths of *T. aestivum* seedlings and the highest values of electrical conductivity and total phenols. The third day of incubation presented only the fourth major reduction in the radicle length of *T. aestivum* seedlings and presented the lowest electrical conductivity for the biomass + soil treatment.

The soil underwent few modifications, but the most noticeable occurred between the first and the seventh days of incubation. From the seventh day, the chromatograms appeared very similar to each other, although slight and practically unnoticeable differences between the seventh and 21st days of degradation were observed (Figure 6). However, the inhibitory effect of the extracts occurred until the 10th and 14th days of incubation, which reinforces the hypothesis that the extraction method was not adequate for detecting the bioactive compounds present in the extracts.

The longest radicle lengths of *T. aestivum* seedlings were recorded between the first and seventh days of soil incubation. The first, third, and seventh days of incubation presented total phenol values of 1,156, 863, and 641 mg $L^{-1}$, respectively, which are much higher than the values observed on the 10th, 14th, and 21st days of soil incubation (61, 104, and 318 mg $L^{-1}$, respectively). These results suggest a tendency of an inverse relation between electrical conductivity and *T. aestivum* radicle length.

The combination of biomass and soil increased the phytotoxicity compared with biomass alone, even despite the ability of allelochemicals to be adsorbed, broken down, and inactivated by the soil. Because of the unusual relation between plant residue and soil, additional studies can focus on this relation in

order to understand the behavior of chemicals released due to tissue decomposition and the role of soil in the decomposition process or in the transformation of the released chemicals into more phytotoxic substances. These additional studies should also consider testing the effects of ions on known *E. plana* allelochemicals.

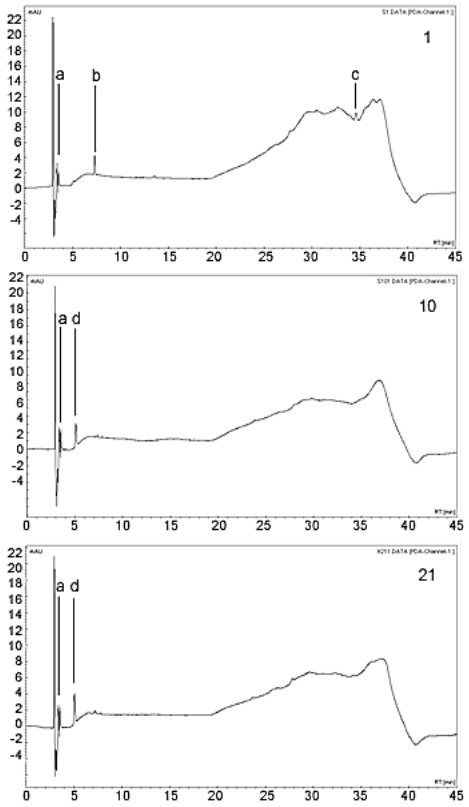


**Figure 6 -** Chromatograms from ethereal extracts of soil incubated for: (1) one day; (10) ten days, and (21) twenty-one days. Chromatograms were obtained using the HPLC technique (High Performance Liquid Chromatography), where Y is the milli-Absorbance Units (mAU) at a given time in minutes (RT) X.

## Conclusion

The highest phytotoxicity of *T. aestivum* seedlings was observed in the extracts obtained from the biomass + soil treatment. The soil treatment was the only one that did not cause phytotoxicity to *T. aestivum* seedlings throughout the incubation period.

Electrical conductivity is an important indicator of the presence of biomass in the aqueous extracts. The presence of biomass caused the electroconductivity values to be more than 100 times higher than those observed in the soil alone at some periods of incubation.

The determination of total phenols is not directly correlated with the phytotoxicity of the aqueous extracts of *E. plana* and thus is not a parameter that explains the phytotoxic effects on the development of *T. aestivum* seedlings.

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