DynamiC of Indole Alkaloids in a Soil and its Relationships with AlleloPathic Properties

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ABSTRACT

Allelopathy is one of the alternatives used for integrated weed management (IWM), in order to minimize the use of synthetic herbicides. Allelopathy is defined as the effect produced by a chemical released by a donor plant on the development of another competitive recipient plant.

Research on allelopathic interactions has been focused on agricultural crops, and allelopathic activity of indole alkaloids has been reported in cereals such as barley (Hordeum vulgare L.), whose principal natural secondary metabolite is Gramine. The degradation products of this metabolite in agricultural soils have not been investigated, however indole and derivatives substituted in aromatic ring and heterocyclic can be produced, so the role of soil in the allelopathic behavior of these compounds is not yet clear.

In this work, phytoxotoxicity and dynamics of Gramine, indole and model series of substituted indoles in position 2 and 3 of the aromatic ring and position 5 of the aromatic ring were investigated in order to understanding the role of this metabolite in the allelopathy property of cereal barley.

The phytotoxic activity against competitive cereals and weeds was determined in soil, from which the percent inhibition (%I) of seed germination and seedling growth was measured. In adsorptions study, according to the values obtained from the adsorption coefficient (Kd), it was obtained that all the series of indole alkaloids shows a moderate adsorption in Alhue soil, with the exception of indole 3-acetic acid. In the study of desorption, this compound showed a desorption percentage of 81%, according to the Kd values obtained. The persistence studies indicated a half-life (t1/2) with a range of values between 7 h and 18 days for the series of indole alkaloids studied, where the highest value of t1/2 was for indole 2-carboxylic acid and the minor one for indole 5-carbaldehyde.

The dynamics of the compounds in Alhue soil affect phytotoxic activity, as well as bioavailability, so that soil plays an essential role in the phytotoxic effect of the compounds. Indole and Indole 2-carboxylic acid had the greatest phytotoxic effect, this behavior can be attributed to its greater persistence and low adsorption, that is, they are more bioavailable in Alhue soil and so the role as an allelochemical would be favorable.

Keywords: barley, allelopathy, indole alkaloids, Gramine, soil, HPLC, sorption, persistence.

1. INTRODUCTION

In cropping systems, weeds are the first factor affecting both crop yield and quality [1] estimated the annual global economic loss caused by weeds at more than 100 billion dollars. In order to achieve the maximization of yields, modern agriculture indiscriminately adopted synthetic chemicals to eliminate the presence of weeds, accompanied by consequent ill effects on soil, water, humans and animal health, as well as an increasing incidence of resistance in weeds [2].

Therefore, the search for alternative weed management strategies more economically and environmentally sustainable is by now of central importance. A natural and environment-friendly strategy for weed control is provided by the manipulation of allelopathic mechanisms between crops and weeds. Allelopathy refers to direct or indirect, harmful or beneficial, effects by a donor plant (including microorganisms) on a target species through the production of chemical compounds that escape into the environment [3].

These compounds, known as allelochemicals, are good candidates for the development of bio herbicides [4]. Allelopathy is a process whereby plants provide themselves with a competitive advantage by putting phytotoxins into the near environment [5]. Evidence has accumulated that crop allelopathy has potential for weed management. The application of crop allelopathy in weed suppression involves two crop growth stages, i.e., vegetative and postharvest. Both growth stages can be employed to suppress weeds. The potential of crop residue allelopathy for weed suppression has been reviewed [3, 6].

Research also has been focused on the utilization of crop seedling allelopathy at the vegetative growth stage, when competition between weeds and crops can take place. The involvement of natural exudates released by crop seedlings at this stage may be critical in determining the natural balances between crop and weed. If a weed species can be allopathically suppressed by crop plants during the seedling establishment period, crop plants will gain an advantage over weeds subsequently.

Plant seedlings of various crops possess allelopathic potential or weed-suppressing activity, including cucumber (Cucumis sativus L.) [7], oat (Avena spp.) [8] and rice (Oryza sativa L.) [9,10]. The main barley allelochemicals are the alkaloids gramine and hordeine [11] which play a significant role in barley allelopathic potential and its defense against weeds, insects, or pathogens [11, 12]. In particular, barley allelopathic extracts have the ability to reduce emergence and growth of serious weeds like winter wild oat (Avena sterilis L.), hood canary grass (Phalaris paradoxa L.), blackgrass (Alopecurus myosuroides Huds.), great brome (Bromus diandrus Roth.), and wild mustard (Sinapis arvensis L.) [13-15]. This growth reduction has been mainly attributed to lipid peroxidation [14, 16, 17].

However, because of this allelopathic potential, some barley varieties could cause essential adverse effects in crop rotations [18, 13], as barley allelopathic potential varied between varieties [19, 15]. Barley has been rated as one of the most important cereals because of its great adaptability to a marginal environment such as dry or saline soils [20].

Because of the stress tolerance expressed by many of its genotypes, barley is cultivated worldwide in soils characterized by high salinity or prolonged drought. Among barley genotypes, varieties with high allelopathic potential would be also appropriate for organic or integrated production systems, where herbicide usage may be limited [21]. Although barley allelopathic varieties are cultivated in marginal soils for suppressing weeds, the effects of stresses like soil salinity on the barley secondary metabolites profile have not been investigated; one major difficulty of in planta assays with purified allelochemicals is to simulate physiologically relevant conditions. In particular; to make assumptions on the allelopathic potential of a compound the concentrations applied in the experiment should reflect those that realistically occur in natural environments.

The allelopathy of an allelochemicals depends on the target species, dose, structure and physicochemical properties. Both, biotic and abiotic factors can trigger the allelopathic potential of a plant. The effectiveness of allelochemicals is therefore considered to be highly dynamics.

Allelopathic compounds are often bound to soil particles; moreover, many of them are hydrophobic. Both these factors potentially increase their biological activity, because retention by soil particles might lead to higher accumulation in the rhizosphere, and because lipophilic compounds might enter root cells more easily. However, association with soil particles and low solubility in water impedes the correct assessment of allelochemical concentrations in soil.

These secondary metabolites are not used by plants in their normal or main metabolism and are present in practically all plant tissues: leaves, stems, roots, rhizomes, flowers, fruits and seeds [22, 23] therefore, allelopathic phenomena can be studied from living plants, where they are exuded into the environment by the roots, or from decomposed material (stubble).

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The allelopathic phenomenon is considered a highly complex process, since it involves various aspects such as: dose, selectivity, structures, and stability of soil, among others. There are three essential factors that need to be investigated to understand the allelopathic phenomenon:

- Determine the selectivity of the allelochemical with respect to its toxicity in cultivars and competitive weeds.
- Characterize the role of the physicochemical properties of the soil and biota, when the allelochemical is released into the rhizosphere.
- Characterize the chemical stability of the allelochemical in the soil and the role of the possible decomposition products in the allelopathic phenomenon.

Numerous studies are known that relate cereal allelopathy with the presence of these allelochemicals in plants. Thus, for example, hydroxamic acids and their decomposition products would be responsible for the allelopathy of corn, wheat and rye [23, 24-26]. The allelopathy of rice and sorghum is attributed to the presence of phenolic acids [6, 27, 28]. Indole alkaloids would be responsible for the barley allelopathy [29, 30].

Simple indole alkaloids, such as gramine, tryptamine, hordeine and derivatives substituted in the aromatic ring (figure 1), are present in different species of grasses, legumes and other families. They cause deleterious effects in mammals, insects, fungi and bacteria [31-32]. Phytotoxic properties of these compounds have also been described in cereals and competitive weeds [27, 30-33-35]. Gramine the main indole alkaloid present in barley shows the highest phytotoxicity.

![Image](https://via.placeholder.com/150)

**GRAMINE:** \( R_1 = R_2 = CH_3; \ n = 1 \)

**INDOLES**

- **TRIPTAMINA:** \( R_1 = R_2 = H; \ n = 2 \)
- \( R = COOH, CHO \)
- \( R = COOH, CHO, CH_2-COOH \)
- \( R_1, R_2, R_3 = H \)

**Figure 1.** Structures of indole alkaloids present in plant and indoles substituted in the aromatic ring and heterocyclic ring.

When an allelochemical is released to the soil, it can be degraded by biotic as well as abiotic effects, so its accumulation and concentration can change rapidly. For this reason, the evaluation of phytotoxic activity under controlled laboratory conditions is often questioned as evidence to explain allelopathic phenomena. The need to understand aspects such as chemical stability, persistence and bioavailability are essential to rationalize these phenomena.

The effect of the rhizosphere on the dynamics of natural indole alkaloids is unknown. However, it is reasonable to expect that the transformation of these compounds will generate substituted indole residues on the aromatic ring and heterocyclic ring (figure 1) as structures closer to the original alkaloids.

Consequently, an in-depth understanding of the role of allelochemicals, as well as their dynamics in agricultural soils, is essential to contribute to the better management of agricultural strategies such as crop rotation or zero tillage.

Thus, the purpose of this study was to assess the alterations in the phytotoxicity of the indole alkaloids (figure 1) using four cereal species target and a competitive weed on an agricultural soil model.

### 2. MATERIALS AND METHODS

#### 2.1 Soil samples

Samples of Alhue soil from VI region, General Libertador Bernardo O’Higgins, Chile, was used in this study. Samples of soil (0-10 cm) were air dried and sieved (2 mm) before using. The physical and chemical properties of soil were determined by described methods [36].

#### 2.2 Reagents

Solutions of Gramine, Indol, Indoline, Indol 2 – carboxyl acid, Indol 3 – carboxyl acid, Indol 5, carboxyl acid, Indole 3 - carboxaldehyde, Indole 5 - carboxaldehyde, Indol 3 – acetic acid were purchase from Aldrich or Merck Chemical, 98%. Solutions were prepared in deionized water (Milli-Q-grade) in a concentration of 100 and 250 mgL\(^{-1}\).

#### 2.3 Phytoxic activity of the compounds.

**2.3.1 Seed germination and seedling grow**

The phytotoxicity of the compounds involved in allelopathic effects depends on the species, dose and destination. For this reason, the phytotoxic activity in the germination development of cereal seeds (Avena – Avena sativa, Wheat - Triticum aestivum, Barley - Hordeum vulgare, Maize - Zea mays,) and a weed Ballica - Lottium rigidum, was evaluated.

For the soil tests, 20 ± 0.1 g of Alhue soil was mashed into petri dishes (five petri dishes for each test and five control petri dishes), these were irrigated with 10 mL of the solution concentration 250 mgL\(^{-1}\) or 100 mgL\(^{-1}\) and the control with 10 mL of Milli-Q grade deionized water, the seeds were uniformly placed and then covered with 5 ± 0.1 g of Alhue soil. Finally, it is sprayed with 2.5 mL of Milli-Q grade deionized water. And the petri dishes were placed in a culture chamber (25 ± C, 12:12 hour’s photoperiod).

**2.4 Sorption-desorption experiments**

Once the equilibrium time was determined, the adsorption process was studied through experiments in Batch. Adsorption of compounds in soil was performed by weighing 1.0 g of soil Alhue. A set of 11 polyethylene bottles was prepared, to which 1.000 ± 0.001 g of the soil samples, then was added 10 mL of 0.01M CaCl\(_2\), solution and zero of the compounds solutions, labeled as bottle 1, up to 0 mL of 0.01M CaCl\(_2\) and 10 mL of the compounds labeled as bottle 11. Samples were stirred at 100 rpm with an orbital shaker, during 48 hours, time determined previously. After stirring, the entire supernatant was transferred to 15 mL centrifuge tubes, which were centrifuged at 3500 rpm for 15 min. After time, the supernatant is filtered with 0.22 μm pore PVDF filters and stored refrigerated for further HPLC analysis. The study was done in duplicate.

For desorption analysis, wet soils were dried at room temperature for one day, and then transferred using the same containers used for adsorption. Then, 5 mL of CaCl\(_2\) 0.01 M solutions was added and stirred for one hour. Afterwards, the solution was centrifuged for 15 min at 3,500 rpm to separate the solid (discarded) from the supernatant. Finally, this supernatant was filtered through a 0.22 μm PVDF membrane.

**2.4.1 Sorption parameters**

Soil sorption was characterized by the partition constant (kd) mL/μg) [37, 38]. The adsorption coefficient (kd) was defined by Equation 1.

\[ kd = \frac{Cs}{Ce} \] (eq. 1)

Where Cs is the concentration of adsorbed compounds in solid phase of soil and Ce is the concentration of the compound in liquid phase of the soil.

The linear or distribution coefficient (Kd) is related to soil organic carbon (OC) and soil organic matter (OM) by the following equations [39, 40].

\[ Koc = 100 \frac{Kd}{\%OC} \] (eq. 2)

**2.5 Persistence.**

The study of persistence consists in determining the concentration of the compound as a function of time elapsed. The persistence times studied were at: 0 - 3 - 6 and 18 hours - 1 - 2 - 3 - 7 - 15 - 21 - 28 - 35 - 48 - 56 and 64 days, in duplicate and control for each time. For the incubation of the soil with the compounds, plastic cups were prepared, 5 g of Alhue soil were weighted and 2.5 mL of each compound solution prepared of 250 mgL\(^{-1}\), were added and 2.5 mL of deionized water to the controls degree Milli-Q, performed in duplicate. These samples remained for the respective times in the culture chamber, at constant temperature and humidity conditions.
The extractions were carried out according to the indicated times, where the soil is extracted quantitatively from the vessels into square flasks, using 10 mL of water / acetonitrile (50/50). The flasks were shaken on an orbital shaker at 100 rpm for one hour, then the entire content is poured into 15 mL centrifuge tubes, centrifuged at 3500 rpm for 15 min and finally the supernatant was extracted with a 5 mL syringe and Transfer to a new 15 mL centrifuge tube using a 0.22 μm PDVF filter. The tubes were kept for analysis.

2.5.1 Half-life time (t1/2)

To determine the persistence of a compound in the soil, the half-life time (t1/2) is determined, which is the time necessary for the dissipation of half the amount of the compound initially present in the matrix. The half-life can be calculated by the following equation:

\[ \text{Ln } (\text{Ce}) = \text{Ln } (\text{Co}) - \text{kt} \]  
(eq. 3)

\[ t_{1/2} = \frac{\text{Ln } 2}{k} \]  
(eq. 4)

Where k is the disappearance constant, which can be calculated using the linear regression equation of Ln Ce versus the incubation time (t).

2.5.2 Leaching

The possible leaching of the compounds can be determined using the GUS index (Groundwater Ubiquity Score), which is an indicator of potential contamination based on an empirical approach.

This index allows compounds to be classified as leachable (GUS > 2.8), non-leachable (GUS < 1.8) and transitional (1.8 < GUS < 2.8) [41].

\[ \text{GUS} = (4 - \log K \sigma c) (\log t_{1/2}) \]  
(eq. 5)

2.6 Analytical method validation and quality control

The determination of compounds was carried out with the help of a high performance liquid chromatography device with diode array detector (HPLC - PDA) WATERS 1525 BINARY HPLC Pump. Column: Atlantis C18, 5.0 μm (Waters); Detector: Photodiode Arrangement, PDA (Waters 2996); Injection volume: 20 μL; flow 1.0 mL / min.

The concentration ranges were based on the limit of detection (LOD) and the limit of quantification (LOQ) for each compound.

Table 1. Analytical parameters of compounds studied, obtained by HPLC analysis.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>R²</th>
<th>% CV</th>
<th>Recovery (%)</th>
<th>LOD mgmL⁻¹</th>
<th>LQD mgmL⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gramine</td>
<td>0.99</td>
<td>1.60</td>
<td>1.55 1.40</td>
<td>105</td>
<td>0.061</td>
</tr>
<tr>
<td>Indole</td>
<td>0.99</td>
<td>0.34</td>
<td>0.20 0.28</td>
<td>102</td>
<td>0.009</td>
</tr>
<tr>
<td>Indol 2-carboxylic acid</td>
<td>0.99</td>
<td>2.42</td>
<td>2.22 2.11</td>
<td>106</td>
<td>0.006</td>
</tr>
<tr>
<td>Indol 3-carbaldehyde</td>
<td>0.99</td>
<td>3.32</td>
<td>0.52 2.44</td>
<td>102</td>
<td>0.009</td>
</tr>
<tr>
<td>Indol 3-carboxylic acid</td>
<td>0.99</td>
<td>2.15</td>
<td>3.43 2.37</td>
<td>97</td>
<td>0.082</td>
</tr>
<tr>
<td>Indol 3-acetic acid</td>
<td>0.99</td>
<td>3.10</td>
<td>2.91 4.35</td>
<td>102</td>
<td>0.042</td>
</tr>
<tr>
<td>Indol 5-carbaldehyde</td>
<td>0.99</td>
<td>1.77</td>
<td>4.85 3.85</td>
<td>100</td>
<td>0.088</td>
</tr>
<tr>
<td>Indol 5-carboxylic acid</td>
<td>0.99</td>
<td>1.41</td>
<td>4.76 4.00</td>
<td>101</td>
<td>0.018</td>
</tr>
</tbody>
</table>

Analytical curves were obtained with a correlation coefficient (R) equal or close to 1 (R² > 0.999). The calibration curves for all the studied elements were in the range of 0.01 to 1.0 mg L⁻¹. The accuracy was measured in relation to coefficient of variation (CV), finding that for all the measurements performed in different days the CV was less than 5%. The method showed good accuracy, with recovery values between 97 and 105 in relation to the standards.

2.7 Determination of lipophilicity.

For the determination of lipophilicity, an alternative HPLC method to the traditional octanol-water (Kow) method was performed. The capacity factor (K') was determined by the RP-HPLC method, since a good correlation is frequently found between the K' and Kow values of organic compounds.

RP-HPLC analyzes are performed on C18 columns with water mobile phase (pH = 3.0 phosphoric acid) / acetonitrile 40:60 v/v.

The capacity factor for the compounds in this study was determined from the following equation:

\[ k' = \frac{(t_R - t_M)}{t_M} \]  
(eq. 6)

Where tR is the retention time of the compound and tM is the retention time of the untrained compound, corresponding to Thiourea.

The correlation between the K' values obtained and the Kow values reported for some of the compounds allows an expression to be derived to obtain logP_HPLC values for the compounds used.

3. RESULTS AND DISCUSSIONS

3.1 Phytotoxic activity of the compounds

The phytotoxicity of a chemical compounds depends, among others, on the species, dose, destination, structure, stability, physical-chemical properties, environmental conditions.

The toxicity of an herbicide can have any of the following effects: Toxicity in the germination of seeds and development of the radicle of monocots or dicots; inhibitory effect on the energy metabolism of chloroplasts and mitochondria; modifications of the binding affinity of the receptor site in membranes.

Due to all these factors, the evaluation of the phytotoxic activity of a chemical is considered a highly complex process [42].

One of the most important and critical stages in plant growth, is the germination. The length of the seedling and fresh weight are also greatly by concentration of the allelochemicals [43, 44]. Therefore, the toxicity in seed germination and development of seedlings using an agricultural model soil

3.1.1 Seed germination and seedling growth in laboratory soil bioassays.

As indicated above, the soil can play an essential role in the phytotoxic activity of an allelochemical. On the other hand phytotoxic effects depend on the dose and target species. Therefore, the selectivity of the series studied was evaluated in 4 species of cereals and a competitive weed using the model Alhue soil treated with two concentration solution (250 and 100 μg mL⁻¹) of compounds under controlled laboratory conditions.

Table 2 shows effect of the compounds on the germination of seeds with respect to the control. The effect was diverse according to the structure and target species.
Table 2. Percentage of growth inhibition and stimulations (+ values) of seeds germination in soil Alhue.

<table>
<thead>
<tr>
<th>COMPOUNDS</th>
<th>Concentration (µgmL⁻¹)</th>
<th>Lolium rigidum L.</th>
<th>Hordeum vulgare L.</th>
<th>Avena sativa</th>
<th>Triticum durum</th>
<th>Zea Mays L.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gramine</td>
<td>250</td>
<td>50.00</td>
<td>0.00</td>
<td>46.67</td>
<td>17.39</td>
<td>ND</td>
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<td></td>
<td>100</td>
<td>12.50</td>
<td>0.00</td>
<td>20.00</td>
<td>13.04</td>
<td>ND</td>
</tr>
<tr>
<td>Indole</td>
<td>250</td>
<td>71.43</td>
<td>5.14</td>
<td>29.41</td>
<td>11.11</td>
<td>ND</td>
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<tr>
<td></td>
<td>100</td>
<td>57.14</td>
<td>5.00</td>
<td>11.76</td>
<td>5.36</td>
<td>ND</td>
</tr>
<tr>
<td>Indole 2-carboxylic acid</td>
<td>250</td>
<td>17.65</td>
<td>6.67</td>
<td>5.56</td>
<td>40.00</td>
<td>46.15</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>+49.09</td>
<td>6.15</td>
<td>5.56</td>
<td>39.13</td>
<td>33.33</td>
</tr>
<tr>
<td>Indole 3-carbaldehyde</td>
<td>250</td>
<td>0.00</td>
<td>4.67</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>100</td>
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<td>+7.69</td>
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<tr>
<td>Indole 3-carboxilic acid</td>
<td>250</td>
<td>+7.69</td>
<td>9.09</td>
<td>10.53</td>
<td>5.56</td>
<td>+8.33</td>
</tr>
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<td></td>
<td>100</td>
<td>+13.64</td>
<td>20.00</td>
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<td>0.00</td>
<td>12.50</td>
</tr>
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<td>Indole 3-acetic acid</td>
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<td>+9.09</td>
<td>0.00</td>
<td>0.00</td>
<td>11.11</td>
</tr>
<tr>
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<td>Indole 5-carbaldehyde</td>
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<td>Indole 5-carboxylic acid</td>
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<td>+20.00</td>
<td>11.76</td>
<td>16.00</td>
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<td>+10.00</td>
<td>23.53</td>
<td>3.70</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

ND: No determined. (+) Stimulating effect. Gramine and Indole values previously reported [45].

In general, gramine and Indole showed the higher inhibitory activity on the weed *Lolium rigidum L.* and the cereal *Avena sativa*. Indole 2, carboxylic acid showed the higher inhibitory activity on the cereals *Triticum durum* and *Zea Mays L.* The compounds substituted in the position 5 of aromatic ring and position 3 of the heterocyclic ring showed a stimulating effect (+ values) on the germination. The weed *Lolium rigidum L.* at the concentration of 100 µgmL⁻¹, Indole 3-carbaldehyde and indole3-acetic acid also showed stimulating effect on the others species studied.

The effect of the compounds in the development of the seedling with respect to control is shows in table 3.

Table 3. Percentages of inhibition and stimulation (+ values), of seedling growth in Alhue soil.

<table>
<thead>
<tr>
<th>COMPOUNDS</th>
<th>Concentration (µgmL⁻¹)</th>
<th>Lolium rigidum L.</th>
<th>Hordeum vulgare L.</th>
<th>Avena sativa</th>
<th>Triticum durum</th>
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<tr>
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<td>38.99</td>
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<td>76.12</td>
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<td>41.73</td>
<td>23.98</td>
<td>ND</td>
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<td>100</td>
<td>64.25</td>
<td>29.62</td>
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<td></td>
<td>100</td>
<td>+16.76</td>
<td>0.00</td>
<td>7.51</td>
<td>+36.82</td>
<td>27.88</td>
</tr>
<tr>
<td>Indol 3-acetic acid</td>
<td>250</td>
<td>3.29</td>
<td>+23.24</td>
<td>+1.45</td>
<td>12.28</td>
<td>+21.99</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>8.02</td>
<td>+53.48</td>
<td>+21.58</td>
<td>+61.34</td>
<td>38.46</td>
</tr>
<tr>
<td>Indol 5-carbaldehyde</td>
<td>250</td>
<td>17.00</td>
<td>4.58</td>
<td>30.96</td>
<td>8.75</td>
<td>38.23</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>13.10</td>
<td>+13.35</td>
<td>13.06</td>
<td>6.93</td>
<td>+30.27</td>
</tr>
<tr>
<td>Indol 5-carboxylic acid</td>
<td>250</td>
<td>9.30</td>
<td>+61.46</td>
<td>63.30</td>
<td>8.74</td>
<td>13.27</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>13.30</td>
<td>+77.56</td>
<td>49.22</td>
<td>6.93</td>
<td>43.09</td>
</tr>
</tbody>
</table>

ND: Not determined; (+) Stimulating effect. Gramine and Indole values previously reported [45].
In contrast with the observed on the seeds germination all the compounds showed inhibition effect on the weed *Lolium rigidum* L. Particularly relevant are the inhibition effects produced by indole 2 carboxylic acid on the species *Triticum durum* and *Zea Mays* L. Indoles-5-carboxylic acid showed a high stimulation effect (+ values), on *Hordeum vulgare* L. and a high inhibition effect on *Avena sativa* at the two concentrations used.

How in the seeds germination, stimulation and/or inhibit activity on the development of the seedling according with the concentration used were observed. In general, indole shows the higher percentages of inhibition on the seedling growth on all the species studied.

### 3.2 Determination of lipophilicity

The lipophilic parameter is essential in the dynamics of a chemical in the physiological and environmental environment, since the hydrophilic-lipophilic balance is fundamental in the distribution of bioactive species in media with different physicochemical characteristics. This physicochemical parameter is used for the prediction of the distribution between environmental compartments, to estimate bioaccumulation in animals and plants and in the prediction of the toxic effects of a substance in quantitative structure-activity relationship (QSAR) studies [46-48].

The lipophilicity of a compound provides direct information on hydrophobicity that describes the tendency of the distribution of a solute from the aqueous phase to the organic components of the environmental compartments and even in biological membranes [48-50]. Therefore, lipophilicity could determine the distribution of the allelochemical in the plant and the dynamic behavior in soil.

In this study, the lipophilicity of the compounds was determined from the HPLC method, alternative to the traditional octanol / water method. The relationship between the capacity factor $K'$ and Kow values was established by a regression between the $K'$ values and LogKow values reported in the literature for some of the compounds. For this, the following $K'$ values, obtained in the literature, were used.

### Table 4. Capacity factor ($K'$) and octanol-water partition coefficient for indole derivative compounds.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>$K'$</th>
<th>Log$K'$</th>
<th>LogKow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gramine</td>
<td>0.21</td>
<td>-0.67</td>
<td>1.45</td>
</tr>
<tr>
<td>Indole</td>
<td>0.89</td>
<td>-0.05</td>
<td>2.10</td>
</tr>
<tr>
<td>5-Metanol indole</td>
<td>0.18</td>
<td>-0.74</td>
<td>1.40</td>
</tr>
<tr>
<td>Hydrox-5-indole</td>
<td>0.64</td>
<td>-0.19</td>
<td>2.00</td>
</tr>
</tbody>
</table>

From these data, a linear regression $R^2 = 0.996$ was performed, from which a predictive expression of the LogP*HPLC* values for the indole compounds was derived:

$$y = 1.0571x + 2.1744 \quad (\text{eq. 7})$$

Where $y$, is equivalent to LogP*HPLC*, $x$, is equivalent to Log $K'$.

With the $K'$ values found for each compound, the LogP*HPLC* values were calculated, which are shown in the table 5, in addition, the values for Gramine and Indole previously determined are included [45].

### Table 5. LogP*HPLC* values obtained for each compounds.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>LogP<em>HPLC</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Gramine</td>
<td>1.45</td>
</tr>
<tr>
<td>Indole</td>
<td>2.10</td>
</tr>
<tr>
<td>Indoline</td>
<td>1.91</td>
</tr>
<tr>
<td>Indol 2-carboxylic acid</td>
<td>1.64</td>
</tr>
<tr>
<td>Indol 3-carbaldehyde</td>
<td>1.88</td>
</tr>
<tr>
<td>Indol 3-carboxylic acid</td>
<td>1.80</td>
</tr>
<tr>
<td>Ac. Indol 3-acetic acid</td>
<td>1.37</td>
</tr>
<tr>
<td>Indol 5-carbaldehyde</td>
<td>1.70</td>
</tr>
<tr>
<td>Indol 5-carboxylic acid</td>
<td>1.45</td>
</tr>
</tbody>
</table>

The lipophilic values fluctuated between 1.3 and 2.1. These values are within the lipophilic range considered “moderate”. The indole compound showed the highest values and indole 3-acetic acid presented the lowest lipophilic value.

Some of the most used herbicides in Chile [51], have moderate lipophilic values, such as: 2, 4 - D (Log Kow = 2.81); Atrazine (Log Kow = 2.61) and Metribuzin (Log Kow = 1.70), on the other hand, some have a high lipophilic character, such as Pendimethalin (Log Kow = 5.18) and Oxyfluorfen (Log Kow = 4, 46) [52].

More clarity about the role of lipophilicity in the phytotoxic activity of the compounds under field conditions was obtained from the relationship between the LogP*HPLC* values and the average percentages of the germination and growth inhibitory activity of the seedlings for the different species determined from the soil matrix are showed in figure 2.

### Figure 2. Relationship between the LogP*HPLC* values and the average percentages of the germination and growth inhibitory activity (H =indole; Indol 2-carboxylic acid = 2-COOH; Indol 3-carbaldehyde = 3 CHO).

#### 3.3 Study of the dynamics of characters in the Alhue soil.

The activity of the allelochemical in the soil would be altered or influenced by soil factors such as organic matter, inorganic ions, reactive mineral surfaces, ion exchange capacity and biotic barriers [53, 54], likewise, could be exposed to various physicochemical and biological processes, where it can detoxify, become more toxic or it can serve as a carbon skeleton for the production of new toxins by soil organisms [55, 56].

The population dynamics of microorganisms in the rhizosphere is largely influenced by available carbon resources, as well as nitrogen, water temperature, soil pH, among others. The compounds under study, when released into the rhizosphere, could become a source of carbon and nitrogen for many microorganisms and could cause the selective induction and/or reproduction of microbes capable of using them [55, 56].

In order to hinder plant growth, allelochemicals must accumulate and persist at phytotoxic levels in the soil, however, after their entry into the environment, the persistence, availability, and biological activities of the allelochemicals are influenced. By microorganisms [54] and by the specific characteristics of the soil, therefore, there is a need to consider soil processes explicitly to make allelopathy research relevant for agricultural systems [53].

The adsorption process of the adsorbate by the adsorbent can be described through adsorption isotherms as favorable, linear or unfavorable, which allows predicting the mobility of the compounds in the soil, which relate the adsorbed mass and the concentration detected in the solution. The adsorption isotherms for the compounds under study are shown in Figure 3.
In all the compounds under study, an isotherm of the S type was obtained; these isotherms are typical of adsorbents with a high affinity for the solvent. The initial direction of the curvature indicates that adsorption is facilitated as the concentration of the compound increases. These isotherms indicate: a specific interaction between the solute and the adsorbent; moderate intermolecular attraction and competition between solute, solvent and adsorbed molecules for specific adsorption sites, except for Indole 3-carbaldehyde and Indole 3-carboxylic Acid which obtained linear adsorption.

3.3.1 Sorption parameters: Kd and Koc.

These coefficients provide information on the interaction between adsorbate and adsorbent; however, in this case as the study will be carried out in the Altue Soil. The distribution coefficient (Kd) represents the mobility of compounds in the soil, that is, the ability of the soil to retain a compound. On the other hand, the adsorption coefficient (Koc) indicates the affinity of the compound with the organic fraction of the soil.

For the determination of these parameters, the values of the measurements of flask 6, of the studied of sorption (batch experiments), which corresponds to the 50/50 proportion of CaCl₂ and the compounds under study, as well as the Eq. 1 and Eq 2.

Table 6. Kd and Koc parameters for each compound.

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>Kd</th>
<th>Koc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gramine</td>
<td>20.98</td>
<td>1676.00</td>
</tr>
<tr>
<td>Indole</td>
<td>0.58</td>
<td>47.40</td>
</tr>
<tr>
<td>Indol 2 carboxylic acid</td>
<td>3.92</td>
<td>194.73</td>
</tr>
<tr>
<td>Indol 3-carbaldehyde</td>
<td>19.66</td>
<td>976.65</td>
</tr>
<tr>
<td>Indol 3-carboxylic acid</td>
<td>2.47</td>
<td>122.70</td>
</tr>
<tr>
<td>Indol 3-acetic acid</td>
<td>1.86</td>
<td>92.40</td>
</tr>
<tr>
<td>Indol 5-carbaldehyde</td>
<td>11.69</td>
<td>580.72</td>
</tr>
<tr>
<td>Indol 5-carboxylic acid</td>
<td>12.43</td>
<td>617.50</td>
</tr>
</tbody>
</table>

The Kd parameter relates the adsorbed concentration versus that found in solution. Indol 3-carbaldehyde presented the highest Kd value, followed by Indol 5-carboxylic acid and indole 5-carbaldehyde, which have higher adsorbed concentration in solid than in solution. The lowest values of Indole indicate the highest mobility of the compounds in soil.

Figure 3. Adsorption isotherms.

According to the classification for Koc [57], Koc values less than 1000 are considered low, where all the compounds have moderate adsorption, with the exception of and Indole 3-acetic acid which are considered weak according to this classification.

Thus then, it could be suggested that those compounds with weak adsorption could be only weakly fixed by the soil components [57], therefore, they could be better distributed in the soil solution.

By relating these values to the values of the bioassay in the soil matrix, it can be inferred that the compounds with high Kd and Koc values, when adsorbed by the soil, present a low germination and seedling development, since they are not bioavailable in the soil, it is observed that the sorption of the compounds under study is lower than of Gramine (1676) and Indole (47.4), in all cases, they widely exceeded to the indole value.

3.3.2 Desorption.

Desorption study was carried out from the adsorption flask 6, which contained a 50/50 ratio of CaCl₂ and the compounds under study. CaCl₂ solution was used as an extracting reagent, which allows maintaining the ionic strength of the solution, stabilizing the charge of the solid particles, and achieving the same level of aggregates in the porous medium [58]. Table 7 shows the values obtained for the study of the desorption of each compound.

Table 7. Study of desorption for each compound.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Conc. added (mg/L)</th>
<th>Conc. found (mg/L)</th>
<th>Desorption (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gramine</td>
<td>500.90</td>
<td>3.46</td>
<td>0.69</td>
</tr>
<tr>
<td>Indole</td>
<td>250.50</td>
<td>26.00</td>
<td>10.64</td>
</tr>
<tr>
<td>Indol 2 carboxylic acid</td>
<td>50.00</td>
<td>7.33</td>
<td>14.67</td>
</tr>
<tr>
<td>Indol 3-carbaldehyde</td>
<td>125.00</td>
<td>1.13</td>
<td>2.26</td>
</tr>
<tr>
<td>Indol 3-carboxylic acid</td>
<td>50.00</td>
<td>34.30</td>
<td>68.60</td>
</tr>
<tr>
<td>Indol 3-acetic acid</td>
<td>125.00</td>
<td>40.58</td>
<td>81.17</td>
</tr>
<tr>
<td>Indol 5-carbaldehyde</td>
<td>125.00</td>
<td>1.00</td>
<td>2.00</td>
</tr>
<tr>
<td>Indol 5-carboxylic acid</td>
<td>50.00</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND: No determined
Indole 3-acetic acid, has the highest desorption percentage (81%), which agrees with the values of Kd (1.8) and Koc (92.4) suggesting that this compound is weakly retained by the soil, as well as the 3-carboxylic indole acid, with values of Kd (2.5) and Koc (122.7) and 2-carboxylic indole acid, with Kd (3.9) and Koc (194.7) values. On the other hand, for 5-carboxylic indole acid, was not obtained a detected percentage of desorption, in which the value of Kd (12.4) and Koc (617.5) indicates that this compound is strongly retained by this soil, therefore it does not desorb. The compounds under study show higher desorption values than Gramine (0.69%), and Indole (10.64%), with the exception of compounds with a CHO substituent on the aromatic and heterocyclic ring.

Desorption plays an important role in the allelopathic phenomenon, since the adsorbed compounds must be desorbed into the soil solution in order to be bioavailable. With the results obtained, it could be inferred that those compounds with a low percentage of desorption will be less bioavailable in the soil, so that allelopathic activity would be affected, as is the case with Indole 3-carbaldehyde, Indole 5-carbaldehyde and Indole 5-carboxylic acid.

3.3.3 Persistence

Persistence studies are important to understand the dynamics of a compound in the soil to determine the persistence or residence time that it could have in it, since this parameter could indicate the period in which the compound will be bioavailable. Next, table 8 shows the study of the persistence of the compounds.

Table 8. Gramine, indole and Indole alkaloids, % values at time zero and at the end of the study.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Concentration (%) Initial time</th>
<th>Duration of the Study</th>
<th>Concentration (%) Final time</th>
<th>Decayment (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gramine</td>
<td>54.28</td>
<td>6</td>
<td>2.50</td>
<td>4.60</td>
</tr>
<tr>
<td>Indole</td>
<td>100</td>
<td>24</td>
<td>9.85</td>
<td>9.85</td>
</tr>
<tr>
<td>Indol 2 carboxylic acid</td>
<td>11.76</td>
<td>672</td>
<td>3.81</td>
<td>32.39</td>
</tr>
<tr>
<td>Indol 3-carbaldehyde</td>
<td>15.49</td>
<td>6</td>
<td>8.00</td>
<td>51.64</td>
</tr>
<tr>
<td>Indol 3 carboxylic acid</td>
<td>16.09</td>
<td>168</td>
<td>6.09</td>
<td>37.84</td>
</tr>
<tr>
<td>Indol 3-acetic acid</td>
<td>14.42</td>
<td>18</td>
<td>3.91</td>
<td>27.11</td>
</tr>
<tr>
<td>Indol 5-carbaldehyde</td>
<td>8.91</td>
<td>24</td>
<td>1.12</td>
<td>12.57</td>
</tr>
<tr>
<td>Indol 5 carboxylic acid</td>
<td>11.94</td>
<td>168</td>
<td>4.52</td>
<td>37.85</td>
</tr>
</tbody>
</table>

It can be seen that for time zero (0 h), the concentrations found are low, which would indicate that the latency period does not occur, that is, immediately in the soil the compound could degrade or be adsorbed by the ground, except for Indole 3-carboxylic indole acid; and 5-carboxylic indole acid, show a similar decay of approximately 38 % in 168 hours (7 days), also the Indole 2-carboxylic acid, shows a decay of 32.39 % in 672 hours (28 days). On the other hand, Indole 3-carbaldehyde presented a 51 % decay in 6 hours, obtaining highest results than Gramine, in Alhue soil, which was not detected beyond 6 hours due to the strong interaction that it would present with the soil. Making its detection and quantification was impossible. Indole 3-acetic acid had a 27.11% decay in 18 hours, similar to Indole, which decayed approximately 10% in 24 hours.

3.3.4 Half-life time

From these results, the half-life time was calculated, obtaining the data presented below.

Table 9. Half-life time values (t_{1/2}) for each compound (eq. 3 and 4).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>t_{1/2}</th>
<th>Gus index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hours</td>
<td>Days</td>
</tr>
<tr>
<td>Gramine</td>
<td>2.00</td>
<td>0.08</td>
</tr>
<tr>
<td>Indole</td>
<td>6.74</td>
<td>0.28</td>
</tr>
<tr>
<td>Indol 2-carboxylic acid</td>
<td>433.22</td>
<td>18.05</td>
</tr>
<tr>
<td>Indol 3-carbaldehyde</td>
<td>6.47</td>
<td>0.27</td>
</tr>
<tr>
<td>Indol 3-carboxylic acid</td>
<td>103.45</td>
<td>4.31</td>
</tr>
<tr>
<td>Indol 3-acetic acid</td>
<td>9.82</td>
<td>0.41</td>
</tr>
<tr>
<td>Indol 5-carbaldehyde</td>
<td>7.82</td>
<td>0.33</td>
</tr>
<tr>
<td>Indol 5-carboxylic acid</td>
<td>169.06</td>
<td>7.04</td>
</tr>
</tbody>
</table>

Compounds with COOH substituents in their structure, presented the highest persistence values in the soil (>100 h), which would indicate that their bioavailability could be higher, on the other hand, compounds with CHO substituents in their structure, presented values low persistence (between 6 h and 7 h), so its bioavailability would be lower.

Since Indole 2-carboxylic acids and Indole 3-carboxylic acids are weak acids, with pKa values of 3.6 and 3.5 respectively, they lose a proton and therefore exist predominantly as anions, mainly in soils whose pH is between 5 and 8, in general, the anions are rejected by the soil surface; therefore the adsorption is usually weak, making them more persistent [59].

3.3.5 Leaching

The main characteristics of the compound that will influence the leaching phenomenon through the soil profile are those that determine the degree of retention of the compound by soil colloids, solubility in water, vapor pressure and hydrophobic character [60].

Taking into account the values (table 9) obtained, compounds with a COOH substituent in their structure can be classified as leachable compounds, which have values greater than 2.8, exceeding the values of Gramine and Indole. On the other hand, the compounds with CHO substituent in their structure, presented low values, which would classify them as non-leachable compounds, like Gramine.

Relating these data to what was obtained in the adsorption study, it can be seen that Indole 3-carbaldehyde, classified as non-leachable, with the lowest GUS value (0.82), has a high Kd (19.66), which confirms that this compound is strongly adsorbed by the soil, for which its leaching is very low. On the other hand, indole 3-carboxylic acid, classified as leachable, with a high GUS value (3.85), has a low Kd (2.47), confirming that by presenting little adsorption in the soil, leaching is greater.

3.5 Phytoxicity - agro-dynamic relationship.

To determine the potential impact on the allelopathic phenomenon, the results obtained from structure - phytoxicity - agro-dynamics relationships were rationalized. This relationship is mainly based on the toxic effect caused by a substance, depending on its composition and the structure of the compound, through quantitative evaluations, relating phytotoxic data and physicochemical properties [61] and agro dynamics allows establishing the bioavailability of the compounds to favor its use as an allelopathic.

With the results obtained in the study of adsorption - desorption, in general it can be inferred that compounds with high Kd and low desorption, such as Gramine and the derivatives indole 3-carbaldehyde, indole 5-carbaldehyde and indole 5-carboxylic acid. They would be less bioavailable and consequently less phytotoxic than those with low Kd and high desorption such as Indole and Indole 2-carboxylic acid. The derivatives indole 3-carboxylic acid and indole 3-acetic acid escape this generality.

The bioavailability relationship in the phytotoxic activity of the compounds in Alhue soil can be observed more clearly, from the relationship between Kd and the average phytotoxic activity, as shown in Figure 4.
From this relationship, the effect of the substrains on the phytotoxic activity can be extrapolated, obtaining an order of phytotoxicity for the carbonyl group: Indole 2-carboxylic acid > Indole 5-carboxylic acid > Indole 3-carboxylic acid. On the other hand, the aldehyde group presented greater phytotoxic activity when being in the 5 position of the aromatic ring than in the 3 position of the heterocyclic ring. In general, the unsubstituted alkaloid, Indole, presented the highest phytotoxic activity, exceeding the values of the natural alkaloid Gramine.

Given that the observed phytotoxicity, as well as the dynamics in the Allue soil of the studied compounds, would be close to those expected under field conditions, these results are relevant to understand the potential relative role of natural indole structures in allelopathic phenomena among competitive plants.

One way to determine the influence of soil characteristics on the behavior of these compounds could be by studying soils with different characteristics, especially pH, OC, content and type of clays, and others.

**CONCLUSIONS**

- The determination of lipophilicity indicates that at higher values of LogPow, the effect on inhibition in seed germination and seedling development is greater.
- The behavior of compounds in a soil is determined by persistence, adsorption / desorption studies. In determining persistence, the series of compounds under study obtained higher half-life values than Gramine and Indole. Regarding adsorption, it is concluded that compounds with high Kd values, being strongly adsorbed by the soil, are less bioavailable, which affects their phytotoxic activity. The results of the desorption study agree with this, since those compounds with high Kd have low desorption values.
- The dynamics of the compounds in the soil affect the phytotoxic activity and their bioavailability, which is why they should be considered to understand more clearly the allelopathic phenomena of indole compounds.
- Taking into account the results obtained in this study: Phytotoxic effect - Persistence - Adsorption, it can be inferred that Indole and Indole 2-carboxylic acid would present a potential allelopathic effect.

**ACKNOWLEDGMENTS**

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**CONFLICT OF INTEREST**

The authors declare that they have no conflict of interest.

**REFERENCES**