Effect of Antibiotics on the Germination and Root Elongation of Argentine Intensive Crops

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Received 18 May 2016; Revised 2 Sep. 2016; Accepted 15 Sep. 2016

ABSTRACT: For small cheese-making factories, with no effluent treatment plants, whey can be a serious problem and the landfarming technique represents an alternative for its disposal. However, whey may contain antibiotic residues at the Maximum Residue Limits. Therefore, in this work, we evaluated the phytotoxic effect of different concentrations of five antibiotics (enrofloxacin, kanamycin, oxytetracycline, penicillin and tylosin) on the germination frequency and root elongation of five Argentine crops: sunflower (Helianthus annuus), corn (Zea mays), soybean (Glycine max), sorghum (Sorghum bicolor), and wheat (Triticum aestivum). We concluded that the antibiotic concentrations currently allowable for the dairy industry (Maximum Residue Limits) are a potential risk for S. bicolor, corn and G. max crops. Results showed that 0.10 mg/l of enrofloxacin affected sorghum, 0.15 mg/l of kanamycin affected corn and G. max, 0.004 mg/l of penicillin affected corn, G. max and S. bicolor, and that 0.05 mg/l of tylosin affected G. max. Therefore, dumping whey contaminated with antibiotic residues on the soil is not recommended.

Key words: Antibiotics, Phytotoxicity, Seeds, Root elongation, Germination frequency

INTRODUCTION
The use of antibiotics in veterinary medicine represents a widespread practice (Ghava et al., 2015). In milk-producing cattle, antibiotics are used to treat mastitis, metritis, pneumonia, enteritis, hoof diseases, etc. (Sawant et al., 2005; Cabello, 2006; Martinez, 2009; Topp et al., 2013). However, the Argentine legislation prohibits the incorporation of milk contaminated with antibiotic residues above the Maximum Residual Limits (MRLs) into the food chain, whereby, this milk must be removed from the market (Argentine Food Code, 2001). In contrast, milk containing antibiotic residues below the MRLs can be processed in the dairy industry and its effluent can be discarded to the environment (European Community, 2009; Codex Alimentarius, 2010). For small cheese-making factories, with no effluent treatment plants, whey represents a serious problem, which can be solved by landfarming (Mannetje et al., 2008). This technique involves the controlled disposal of effluents on the soil to achieve biological and chemical degradation of organic matter (Silva-Castro et al., 2015). However, its application is limited as long times are required for organic matter biodegradation, because it depends on the microbial composition, the physicochemical properties of the soil and the concentration and type of pollutants (Thassitou and Arvanitoyannis, 2001).

In soils under conventional landfarming fertilised with manure and monitored for two years, Wang and Wang (2015) reported average concentrations of up to 199 μg/kg of tetracycline, 7 μg/kg of chlortetracycline and 11 μg/kg of sulfadimidine. In addition, numerous antibiotic molecules are not metabolised, thus being eliminated in the milk or excreted in the urine and/or feces (Chee-Sanford et al.; 2001; Kumar et al., 2005a,b; Pope et al., 2009; Finley et al., 2013; Topp et al., 2013). These molecules can reach the ground and pass to the groundwater (Pedersen et al., 2003; Aga et al., 2003; Batt et al., 2006; Zhang and Li, 2011) or to watercourses adjacent to livestock production areas (Kolpin et al., 2002; Boxall...
et al. 2003; Boxall et al., 2004; Hanscher et al., 2004; Davis et al., 2006; Batt et al., 2006; Aust et al., 2008; Bowman et al., 2011). Therefore, the growth and development of plants (Jjemba, 2002a,b; Liu et al., 2009), the composition of the microbial flora (Mc Cracken Foster, 1993; Chander et al., 2005) and the growth of some native microorganisms (Varnero et al., 2005) can be modified when antibiotic molecules are introduced in the soil.

When manure contaminated with antibiotic residues is dumped on the ground, resistant strains can develop (Knapp et al., 2010). When microorganisms acquire resistance via gene transfer, resistant microbial diseases affecting animals and humans may appear (Thiele Bruhn, 2003; Andersson and Hughes, 2012; Wellington et al., 2013). Therefore, resistant infections are becoming more frequent (Carlet et al., 2011; Finley et al., 2013).

Environmental damage has been assessed by several authors by means of different techniques, including phytotoxicity studies on plant growth and seed germination (Liu et al., 2009). For example, Hillis et al. (2011) studied the effect of some antibiotics on seed germination and root elongation of lettuce (Lactuca sativa), alfalfa (Medicago sativa) and carrot (Daucus carota). Similarly, Bowman et al. (2011) evaluated the toxicity and reduction in intracellular calcium levels due to tetracycline in Arabidopsis. Chen et al. (2011) analysed single and joint toxicity of chloramphenicol with Hg acting on wheat (Triticum aestivum L.), Chinese cabbage (Brassica campestris L.) and corn (Zea mays L.). These authors report a positive correlation between root elongation inhibition and concentrations of pollutants. Liu et al. (2009) analysed the effect of various antibiotics on the growth of rice (Oryza sativa L.), turmeric (Curcuma sativus L.) and sweet oats (Cichorium endive), while Migliore et al. (1994, 1998) evaluated the phytotoxicity of sulfamethoxine, enrofloxacin and oxytetracycline in millet (Panicum milaieaum), peas (Pisum sativum) and maize (Zea mays L.) plants. Moreover, Jjemba (2002a, b) studied the effect of chloroquine, quinacrine and metronidazole in soybean plants and the soil microbiota. However, no studies have evaluated the potential effects of antibiotics on Argentine widespread crops.

Therefore, the objective of this study was to evaluate the effect of six families of antibiotics (aminoglycosides, beta-lactams, macrolides, quinolones, sulfonamides and tetracyclines) on the germination and root elongation of five Argentine crops (Glycine max, Helianthus annuus, Sorghum bicolor, Triticum aestivum and Zea mays) to assess their possible phytotoxic effect.

**MATERIALS & METHODS**

Antibiotics studied: the following antibiotic molecules, representative of each group, were selected: enrofloxacin (quinolones), kanamycin (aminoglycosides), oxytetracycline (tetracycline), penicillin (beta-lactams), and tylosin (macrolides). To assess the possible impact of landfarming with whey containing antibiotic residuals, concentrations equivalent to their MRLs (European Community, 2009; Codex Alimentarius, 2010) were chosen as minimum values for this study. Based on these threshold values, solutions containing the following concentrations in logarithmic scales were prepared: C_0 = 0 MRL, C_1 = 1 MRL, C_2 = 10 MRLs, C_3 = 100 MRLs and C_4 = 1000 MRLs (MRL_{enrofloxacin} = 0.1 mg/l, MRL_{oxytetracycline} = 0.1 mg/l, MRL_{kanamycin} = 0.15 mg/l, MRL_{penicillin} = 0.004 mg/l, MRL_{tylosin} = 0.05 mg/l).

Selected seeds: Glycine max (51%), Zea mays (28%), Triticum aestivum (9%), Helianthus annuus (3%) and Sorghum bicolor (2%) were selected due to its significant production in Argentine (Rosario Stock Exchange, 2015). Crop seeds were provided by Department of Intensive Cultivation of Faculty of Agricultural Sciences, National University of Litoral (Argentina).

Toxicity study on seeds: the guidelines established by the standard protocol to conduct toxicity tests in terrestrial plants were followed (ASTM, 2003; Liu et al., 2009; Hillis et al. 2011; Bowman et al., 2011; Adomas et al., 2013; Ghava et al., 2015). Before starting the treatment, seeds were stored at 4°C for 1 month. After this time, 1500 experimental units of each culture were selected.

For each seed, five replicates of each antibiotic concentration were performed using polystyrene Petri plates (100 x 15 mm). In each plate, 10 seeds were distributed on Whatman cellulose filter. Subsequently, 10 ml of the antibiotic solutions were added to each plate at each concentration (C_0, C_1, C_2, C_3, C_4; 25 plates/antibiotic and seed).

To prevent dehydration, plates were covered with Parafilm M (Sigma-Aldrich) and placed in an incubation chamber at 25°C for 7 days. For germination control, the acceptability criterion was set at 70% (according to the protocol).

**Relative frequency of seed germination.** The relative frequency of seed germination of each species was

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calculated at 12-hour intervals during a period of seven days (14 controls). The effect of antibiotic concentration on the germination frequency of each seed was analysed. The stepwise option of the multiple logistic regression models was used:

\[ Y = \text{Logistic} \ L_{ijk} = \beta_0 + \beta_1 \ t + \Sigma \beta_{ij} Z_{ij} + \epsilon_{ijk} \]

Where: \( Y = \text{Logistic} \ L_{ijk} \); Logistic function = \( \ln \left( \frac{P_{ijk}}{1-P_{ijk}} \right) \); \( P_{ijk} \) from germinated seed and \( 1-P_{ijk} \); probability of non-germinated seeds. \( \beta_0 \): Intercept. \( \beta_{ij} \): coefficients estimated by the logistic model, \( t \); Effect of time on seed germination (i=12), \( Z_i \); Effect of antibiotic concentration in terms of dummy variable \( (Z_{i1}: C_i=0, C_i=0, C_i=0, C_i=0) ; Z_{i2}: C_i=1, C_i=0, C_i=0, C_i=0) ; Z_{i3}: C_i=0, C_i=0, C_i=0, C_i=0) \); \( \epsilon_{ijk} \): residual error of the model. Statistical calculations were performed using Centurion Stat Graphics® software.

Effect of antibiotics on the root elongation of germinated crop seeds. Seven days after the start of the experiment, the root length of each germinated seed was measured in duplicate. Measurements were made from the top of the primary root to the hypocotyl, using a Vernier caliper (accuracy 0.1 mm).

The effect of antibiotic concentration on root elongation was evaluated by means of ANOVA (Centurion Stat Graphics®). Subsequently, Turkey’s test was applied to evaluate significant differences for each antibiotic concentration.

RESULTS & DISCUSSION

Relative frequency of seed germination: Statistical results (“\( \chi^2 \)” and “\( p \)” value) from the application of the stepwise option of the logistic regression model, which predicts germination frequencies for different antibiotic concentrations, are shown in Table 1. These results show that almost all the seeds analysed were affected by enrofloxacin (except for Zea mays) and penicillin (except Helianthus annuus). The other antibiotics affected a smaller number of seeds (Table 1).

The effects of each antibiotic concentration on the germination frequencies of seeds selected by the stepwise option are shown in Fig. 1. In all the cases, the antibiotics caused a delay in the germination frequencies of seeds, showing a phytotoxic action on crop growth. In addition, the logistic equations are shown in Fig. 1. The time affected the germination frequencies significantly (positive coefficients “\( \beta_0 \)”), while the antibiotic concentration negatively affected the germination frequencies, demonstrating an inhibitory effect (negative coefficients “\( \beta_{ij} \)”).

Fig. 1 show that 0.004 mg/l of penicillin, 1.0 mg/l of enrofloxacin and 10 mg/l of oxytetracycline affected the germination frequency of Glycine max and that 0.1 mg/l of enrofloxacin affected the germination frequency of Helianthus annuus. The germination frequency of Sorghum bicolor decreased with 0.004 mg/l of penicillin and 0.05 mg/l of tylös, values equivalent to their respective MRLs. Higher values of enrofloxacin (1 mg/l) were necessary to affect the germination frequency of this crop. Triticum aestivum seeds were affected by 0.4 mg/l of penicillin, 1 mg/l of oxytetracycline and 10 mg/l of enrofloxacin. Zea mays seeds were more resistant to antibiotic residues, since 100 mg/l of oxytetracycline and 4 mg/l of penicillin were necessary to affect their germination frequency.

With the aim to visualize this inhibitory action of antibiotics, Fig. 2 (1a: treated group and 1b: control protein) show that antibiotic residues affected root elongation, and those seeds affected by 1 mg/l of enrofloxacin and 10 mg/l of oxytetracycline presented a shorter root length than controls.

### Table 1. Relative frequency of seed germination - Statistical results from the application of the stepwise option of the logistic regression model for different antibiotic concentrations

<table>
<thead>
<tr>
<th>Seed</th>
<th>Antibiotic</th>
<th>Time</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \chi^2 ) val</td>
<td>P val</td>
<td>( \chi^2 ) val</td>
</tr>
<tr>
<td>Glicine max</td>
<td>Enrofloxacin</td>
<td>1316</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>Oxytetracycline</td>
<td>812</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>Penicillin</td>
<td>935</td>
<td>0.0001</td>
</tr>
<tr>
<td>Helianthus annuus</td>
<td>Enrofloxacin</td>
<td>971</td>
<td>0.0001</td>
</tr>
<tr>
<td>Sorghum bicolor</td>
<td>Enrofloxacin</td>
<td>876</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>Penicillin</td>
<td>2066</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>Tylös</td>
<td>2219</td>
<td>0.0001</td>
</tr>
<tr>
<td>Triticum aestivum</td>
<td>Enrofloxacin</td>
<td>874</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>Oxytetracycline</td>
<td>1753</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>Penicillin</td>
<td>808</td>
<td>0.0001</td>
</tr>
<tr>
<td>Zea mays</td>
<td>Oxytetracycline</td>
<td>1184</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>Penicillin</td>
<td>1172</td>
<td>0.0001</td>
</tr>
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</table>
### Phytotoxic Effect of Antibiotics

<table>
<thead>
<tr>
<th>Plant Species</th>
<th>Antibiotic 1</th>
<th>Antibiotic 2</th>
<th>Logistic Equation</th>
<th>Control Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Glycine max</em></td>
<td>Enrofloxacin</td>
<td>C1=0.1 mg/l; C2=1.0 mg/l; C3=10 mg/l; C4=100 mg/l</td>
<td>Logistic = -2.5982 + 1.1732 t - 0.9637 C2 - 1.3689 C3 - 1.4047 C4; 76%</td>
<td></td>
</tr>
<tr>
<td><em>Glycine max</em></td>
<td>Penicillin</td>
<td>C1=0.004 mg/l; C2=0.04 mg/l; C3=0.4 mg/l; C4=4 mg/l</td>
<td>Logistic = -4.6345 + 2.3279 t - 2.1365 C1; 92%</td>
<td></td>
</tr>
<tr>
<td><em>Glycine max</em></td>
<td>Oxytetracycline</td>
<td>C1=0.004 mg/l; C2=0.04 mg/l; C3=0.4 mg/l; C4=4 mg/l</td>
<td>Logistic = -4.4990 + 1.8463 t - 1.1233 C3; 82%</td>
<td></td>
</tr>
<tr>
<td><em>Helianthus annuus</em></td>
<td>Enrofloxacin</td>
<td>C1=0.05 mg/l; C2=0.5 mg/l; C3=5 mg/l; C4=50 mg/l</td>
<td>Logistic = -0.8773 + 1.5181 t - 0.6372 C1; 80%</td>
<td></td>
</tr>
<tr>
<td><em>Sorghum bicolor</em></td>
<td>Enrofloxacin</td>
<td>C1=0.004 mg/l; C2=0.04 mg/l; C3=0.4 mg/l; C4=4 mg/l</td>
<td>Logistic = -4.2204 + 5.4411 t - 1.1889 C2; 99%</td>
<td></td>
</tr>
<tr>
<td><em>Sorghum bicolor</em></td>
<td>Penicillin</td>
<td>C1=0.004 mg/l; C2=0.04 mg/l; C3=0.4 mg/l; C4=4 mg/l</td>
<td>Logistic = -3.4981 + 5.1998 t - 0.3971 C1 - 0.5293 C2 - 1.1031 C3 - 1.6016 C4; 98%</td>
<td></td>
</tr>
<tr>
<td><em>Sorghum bicolor</em></td>
<td>Tylosin</td>
<td>C1=0.004 mg/l; C2=0.04 mg/l; C3=0.4 mg/l; C4=4 mg/l</td>
<td>Logistic = -3.4981 + 5.1998 t - 0.3971 C1 - 0.5293 C2 - 1.1031 C3 - 1.6016 C4; 96%</td>
<td></td>
</tr>
<tr>
<td><em>Triticum aestivum</em></td>
<td>Enrofloxacin</td>
<td>C1=10 mg/l; C2=100 mg/l</td>
<td>Logistic = -1.6027 + 2.0111 t - 0.1818 C3 - 0.5373 C4; 81%</td>
<td></td>
</tr>
<tr>
<td><em>Triticum aestivum</em></td>
<td>Penicillin</td>
<td>C1=0.04 mg/l; C2=0.4 mg/l; C3=4 mg/l</td>
<td>Logistic = -4.0095 + 7.4352 t - 1.8249 C2; 99%</td>
<td></td>
</tr>
<tr>
<td><em>Triticum aestivum</em></td>
<td>Oxytetracycline</td>
<td>C1=1.0 mg/l; C2=10 mg/l; C3=100 mg/l</td>
<td>Logistic = -3.3399 + 6.4533 t - 0.7368 C2; 98%</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 1. Relative frequency of seed germination - Effects of antibiotics concentration on the germination frequencies of seeds

group) is presented. This image shows that low concentrations of penicillin (0.004 mg/l) affect germination frequency of Glycine max comparing with the control (free-antibiotic), on second day (1 vs. 4 germinated seeds) and fourth day (4 vs. 8 germinated seeds). By the fifth day, all the seeds have germinated in both groups.

To summarise, it can be established that antibiotic concentrations equivalent to the MRLs cause a phytotoxic effect on the germination rate of some crops, with 0.1 mg/l of enrofloxacin affecting H. annuus seeds, 0.004 mg/l of penicillin affecting G. max and S. bicolor, and 0.05 mg/l of tylosin affecting S. bicolor.

Fig. 2. Effect of 0.004 mg/l penicillin on seed germination frequency of Glycine max: (a) treated group, (b) control group

Effect of antibiotics on the root elongation of germinated crop seeds: The mean values, standard deviations and statistical parameters (“F” and “p” values) calculated with ANOVA for the antibiotics that affected seeds are shown in Table 2. Significant differences obtained by applying Turkey’s test are also presented.

For Glycine max, all the antibiotics tested caused a phytotoxic effect. Kanamycin, penicillin and tylosin showed toxicity at their MRLs (MRL_{kanamycin}: 0.15 mg/l, MRL_{penicillin}: 0.004 mg/l, MRL_{tylosin}: 0.05 mg/l), whereas 1 mg/l of oxytetracycline and 10 mg/l enrofloxacin were necessary to affect root elongation. Regarding
Table 2. Effect of antibiotics on the root elongation of germinated crop seed - Main values, standard deviations and statistical parameters calculated with ANOVA for the antibiotics that affected seeds

<table>
<thead>
<tr>
<th>Seed</th>
<th>Antibiotic</th>
<th>Concentration (MRL)</th>
<th>Value</th>
<th>Value</th>
<th>“F”</th>
<th>“p”</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>MRL</td>
<td>10 MRL</td>
<td>100 MRL</td>
<td>1000 MRL</td>
</tr>
<tr>
<td>Glycine max</td>
<td>Enrofloxacin</td>
<td>2.1±0.1</td>
<td>2.1±0.1</td>
<td>1.9±0.1</td>
<td>1.9±0.1</td>
<td>1.4±0.1</td>
</tr>
<tr>
<td></td>
<td>Kanamycin</td>
<td>1.8±0.1</td>
<td>1.8±0.1</td>
<td>1.5±0.1</td>
<td>1.4±0.1</td>
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<td></td>
<td>Oxytetracycline</td>
<td>3.6±0.2</td>
<td>3.2±0.2</td>
<td>2.9±0.2</td>
<td>2.3±0.2</td>
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<td></td>
<td>Penicillin</td>
<td>2.7±0.2</td>
<td>1.7±0.2</td>
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<td></td>
<td>Tylosin</td>
<td>3.0±0.1</td>
<td>2.5±0.1</td>
<td>2.4±0.2</td>
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</tr>
<tr>
<td>Helianthus annuus</td>
<td>Enrofloxacin</td>
<td>2.3±0.1</td>
<td>2.1±0.1</td>
<td>2.1±0.1</td>
<td>1.7±0.1</td>
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<tr>
<td>Sorghum bicolor</td>
<td>Enrofloxacin</td>
<td>7.4±0.2</td>
<td>6.2±0.2</td>
<td>3.9±0.2</td>
<td>2.8±0.2</td>
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<tr>
<td>Triticum aestivum</td>
<td>Oxytetracycline</td>
<td>6.8±0.2</td>
<td>6.0±0.2</td>
<td>5.3±0.2</td>
<td>1.9±0.2</td>
<td>2.0±0.2</td>
</tr>
<tr>
<td>Zea mays</td>
<td>Penicillin</td>
<td>8.3±0.3</td>
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<td>6.4±0.3</td>
</tr>
<tr>
<td></td>
<td>Enrofloxacin</td>
<td>5.4±0.2</td>
<td>43±0.2</td>
<td>3.9±0.2</td>
<td>2.4±0.2</td>
<td>1.9±0.2</td>
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<td>5.7±0.3</td>
<td>5.1±0.3</td>
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</tr>
</tbody>
</table>

a, b, c: Different subscripts values in the same row indicate significant differences at a level of p<0.05

For Zea mays, inhibitory effects were observed at low concentrations of kanamycin (0.15 mg/l) and penicillin (0.004 mg/l) but at high values of oxytetracycline (10 mg/l) and enrofloxacin (100 mg/l). In summary, oxytetracycline affected the root elongation of all seeds studied. This is in agreement with the results of Hillis et al. (2011), who reported inhibitory effect of oxytetracycline in root elongation of Lactuca sativa, Medicago sativa and Daucus carota. When comparing the germination frequency of seeds (Table 1) with their root elongation (Table 2), we found that the second parameter was more sensitive, as also noted by Ghava et al. (2015) and Basset et al. (2002).

In order to appreciate the impacts of low antibiotic concentrations on the roots elongation, the effect of penicillin (0.004 mg/l) on Glycine max was chosen. In Fig. 3 is displayed that penicillin produced a significant decrease in the length of the roots (Fig. 3a) compared to the control.
to free-antibiotics seeds (Fig. 3b) at the seventh day of treatment.

In summary, our results showed that low concentrations of some antibiotics affect the root elongation of the main Argentine crops: 0.15 mg/l of kanamycin, 0.004 mg/l of penicillin and 0.05 mg/l of tylosin affected Glycine max seeds; 0.1 mg/l of enrofloxacin and 0.004 mg/l of penicillin affected Sorghum bicolor, and 0.15 mg/l of kanamycin and 0.004 mg/l of penicillin affected Zea mays. Therefore, the landfarming technique for disposal of whey contaminated with antibiotics at the MRLs values established by the law should be avoided.

In order to avoid the environmental impact of these antibiotics, heat treatment or photocatalytic processes of contaminated whey should be evaluated. Studies in milk indicate that sterilization process (120°C-20min) produces significant losses in the antimicrobial activity of penicillin (Zorraquino et al., 2008, Roca et al., 2011), kanamycin (Zorraquino et al., 2009), tylosin (Zorraquino et al., 2011), except enrofloxacin (Roca et al., 2010). For degradation of quinolones residues in water, Sirtori et al. (2009) suggest a solar photocatalytic processes.

CONCLUSIONS
Due to the possibility of using the landfarming technique for the treatment of contaminated whey, here we evaluated the effect of different antibiotics on the germination frequency and root elongation of some Argentinean crops. We concluded that values equivalent to the Maximum Residue Limits cause phytotoxic effects. Results showed that 0.1 mg/l of enrofloxacin affects the germination of Sorghum bicolor, that 0.15 mg/l of kanamycin affects Zea mays and Glycine max, that 0.004 mg/l of penicillin affects Zea mays, Glycine max and Sorghum bicolor, and that 0.05 mg/l of tylosin affects Glycine max.

These studies show that antibiotic concentrations acceptable for the dairy industry can cause a decrease in the germination frequency and/or a reduction in the root elongation of Glycine max Sorghum bicolor and Zea mays, widely spread crops in Argentina. Helianthus annuus and Triticum aestivum were more resistant. Therefore, the discharge of effluents from the dairy industry contaminated with antibiotics in the soil constitutes an environmental problem.

In order to improve these findings, other aminoglycosides, beta-lactams, macrolides and quinolones should be investigated. It would also be appropriate to assess potential ecotoxicological effects of antibiotics on other biomarkers such as bacteria (Vibrio fischeri), algae (Chlorella vulgaris, Cyclotella meneghiniana, Lemma gibba, Pseudokirchneriella subcapitata), invertebrates (Artemia salina, Ceriodaphnia dubia, Daphnia magna), and fish (Danio rerio, Lepomis punctatus, Lepomis macrochirus, Oncorhynchus mykiss, Salmo trutta, Salvelinus fontinalis, Salvelinus namaycush) to select the most suitable biosensor to be used in the future to control the environmental impact of antibiotics.

ACKNOWLEDGMENTS
This research work has been carried out as part of the CAI+D’11 Projects (50120110100052 LI, Res. 444/13- List II year 2013, National University of Litoral, Santa Fe, Argentine) and PICT 455-2012 (National
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Agency for Scientific and Technological Promotion) and to Prof. Horacio Inmvinkelried, Intensive Cultivation Department of the Faculty of Agricultural Sciences, National University of Litoral.

REFERENCES


Ecosystems and Environment, plants grown on arable land: a review. Agriculture, Ecosystems and Environment, 93, 267-278.


