

## ARTICLE

## CHANGE IN PHYTOTOXICITY DURING COMPOSTING OF MANURES CONTAINING OXYTETRACYCLINE

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## ABSTRACT



Manure from animal farms contains significant amounts of antibiotics, and fertilizers prepared from this manures may contain residuals of antibiotics which are able to suppress soil microbiota and consequently reduce plant growth. Composting is an effective way to reduce the negative impact of antibiotics in manure on the growth of crops. In this paper, we have evaluated the effect of composting on the phytotoxicity of cow manure spiked with various concentrations of OTC (oxytetracycline) (50 mg kg<sup>-1</sup>, 150 mg kg<sup>-1</sup> and 300 mg kg<sup>-1</sup>). Assessment of phytotoxicity was carried out using oat plants (*Avena sativa* L.), by contact method on the 1st, 30th, 60th and 90th days of composting. Composts were mixed with soil in a ratio of 1:1, 2:1 and 3:1. It was found that during the first 30 days, all composts had significant toxicity (the germination index did not exceed 10% for any mixtures). Afterwards, phytotoxicity decreased in all the mixtures. On the 60th day, the GI (germination index) ranged from 16.7% to 40.9%, with different OTC concentrations causing significantly differing effects. On the 90th day, the GI (germination index) ranged from 29.3% to 106.6%, with no significant difference observable in the samples with various initial concentrations of OTC. This may be due to decomposition of the antibiotic in the soil.

## INTRODUCTION

**KEY WORDS**  
manure, composting,  
antibiotics,  
oxytetracycline,  
phytotoxicity.

The use of animal manures after composting to restore the fertility of agricultural soils is a common practice for farmers around the world [1]. Composting provides an environmentally friendly approach for the transformation of livestock waste into an effective non-toxic and non-pathogenic organic fertilizer. In the process of composting, the presence of a wide range of complex organic compounds promotes the development of a diversity of microbial communities in the soil which, as a result, stimulate various soil functions [2].

However, the widespread use of antibacterial drugs for the treatment and disease prevention of animals can make the use of manure dangerous due to the risk of spreading antibiotics into soil ecosystems [3,4]. It has been found that up to 90% of the prescribed dose of antibiotics consumed is excreted from the animal's body due to weak absorption in the gastrointestinal tract. Recent studies have shown that manure from livestock farms contains high antibiotic residues at levels from several mg kg<sup>-1</sup> or up to several hundreds of mg kg<sup>-1</sup> [5]. The most common antibiotics for the treatment of animals are antibiotics of the tetracycline group [5]. Often, untreated animal waste from livestock farms is used to fertilize soils as a raw material [6]. This can create an increasing potential risk for human health, as well as for ecosystems [4]. The use of manure can depress the activity of soil microorganisms and inhibit the natural processes of soil enzyme production [7]. In addition, residues of antibiotics in the composition of manure can pass from the soil into the crops, thereby increasing the risk to human health [8,9]. Such information on the effects of composts contaminated with antibiotics on agricultural plants is, however, scarce. Thus, it is necessary to treat and neutralize this kind of animal waste before using it for soil fertilization [10].

Recently, many studies have shown that composting is an effective method that significantly reduces antibiotics in animal wastes. For instance, Dolliver with coauthors observed the degradation of monensin and tylosin during manure composting and found that the monensin and tylosin was reduced by 54–76% [11]. Rama swamy with coauthors (2010) found that the content of salinomycin decreased by 99.8% over 38 days of composting [10]. According to Arican with coauthors, (2007) the initial level of oxytetracycline decreased significantly by 95% during the first 6 days of composting of cow manure [12]. Such a significant reduction in the content of antibiotics in the manure is most likely facilitated by certain conditions found during composting such as temperature, humidity and various antibiotic biodegradation processes [11,13]. In addition, a decrease in the content of soluble forms of antibiotics may be due to their adsorption on organic components contained in composts. The addition of organic structural agents such as straw and sawdust to manure in compost production can also affect the reduction of antibiotics [6].

The objectives of this paper were as follows: i) to assess the change in phytotoxicity of cow manure containing oxytetracycline in the process of composting, and ii) to reveal the influence of initial oxytetracycline concentration in the manure on the phytotoxicity dynamics.

## METHODS

The object of the study was the manure of cows which had not been treated with antibiotics, selected on a private farm in the Laishevsky District (Republic of Tatarstan). Straw was added to the manure as a structural agent in a ratio 20:6.5 (w/w). Oxytetracycline (OTC) was chosen for the model experiment, since

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this drug is widely used on livestock in Russia. Three composting mixtures containing 50 mg kg<sup>-1</sup>, 150 mg kg<sup>-1</sup> and 300 mg kg<sup>-1</sup> OTC were prepared. As a compost control, a compost mixture without the antibiotic was used. Composts were incubated at room temperature (20–25 °C).

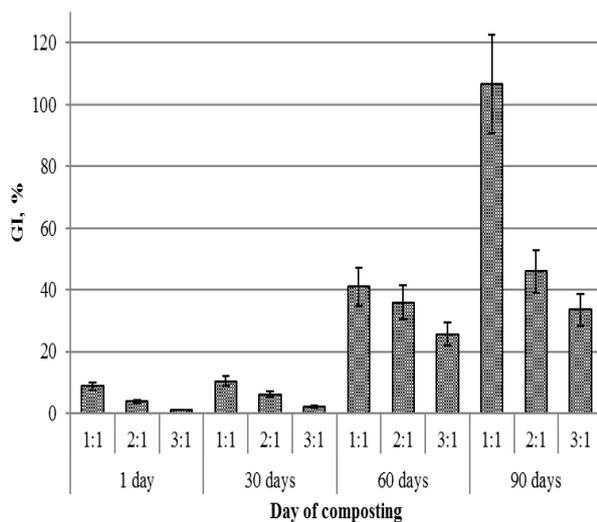
The phytotoxicity of the composts was determined on the 1st, 30th, 60th and 90th days of composting by the contact method using oats (*Avena sativa* L.) as a test object [14]. To do this, composts were mixed with the soil to achieve a 1:1, 2:1 and 3:1 compost–soil ratio. As a soil control, soil without the compost was used. The compost–soil mixtures were moistened up to 60% moisture capacity and maintained at this level throughout the incubation period. Incubation was carried out at room temperature (20–25 °C) for 14 days. The germination index (GI,%) was calculated by the formula:

$$GI (\%) = ((\text{Seed germination in treatment} \times \text{Root length in treatment}) / ((\text{Seed germination in control} \times \text{Root length in control}))) \times 100$$

## RESULTS

Phytotoxicity of composts is one of the often measured parameters characterizing the maturity of composts, and therefore their suitability for safe soil fertilization. This method is based on the evaluation of the effect of compost applied to the soil on the intensity of germination of seeds and the early stages of growth of a number of plants [15]. In this paper, the phytotoxicity of composts contaminated with OTC was investigated using oat (*Avena sativa* L.) seeds.

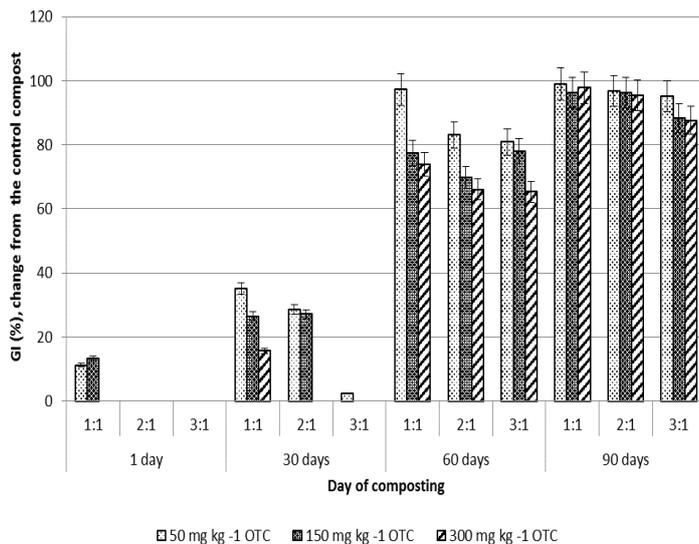
In the first stage, the phytotoxicity of compost–soil mixtures, prepared with manure from cows not containing antibiotics, was analyzed. The results of the estimation of the germination index of the oat seeds (*Avena sativa* L.) are shown on the [Fig. 1].



**Fig. 1:** Change in phytotoxicity in the process of composting of manure not contaminated with antibiotics.

As can be seen from the [Fig. 1], in the first 30rd days of the composting process, all mixtures of soil and compost from manure that did not contain antibiotics had high toxicity. The germination index in this case did not exceed 10%. A correlation was found between the degree of phytotoxicity and the amount of compost mixed with the soil: the higher the compost content, the stronger the inhibitory effect on oat growth. On the 60th day of the composting, the germination index increased to 40.9%, 35.9% and 25.6% in the compost–soil mixtures 1:1, 2:1 and 3:1, respectively. On the 90th day in 1:1 ratio mixture of compost and soil, the germination index was 106.6%, which is 2.6 times higher than that on the 60th day. In mixtures with compost–soil ratios of 2:1 and 3:1, phytotoxicity remained almost unchanged from its level on the 60th day of composting. In general, a trend was observed indicating the reduction of phytotoxicity in compost with the passage of time.

In the second stage of the study, the phytotoxicity of mixtures from soil and compost contaminated with various concentrations of OTC was assessed. The results are shown in [Fig. 2].



**Fig. 2:** Change in phytotoxicity in the process of composting of manure containing different concentrations of OTC.

As can be seen from the [Fig. 2], there were similar dynamics in the change in toxicity in the process of composting of manures contaminated with OTC. During the first 30 days of the composting process all the compost–soil mixtures were characterized by a high level of toxicity.

On the 30th day in the mixture 1:1 there were significant differences in the GI between different concentrations of OTC. On the 60th day there was a decrease in the level of phytotoxicity for all composts. However, significant differences were found between the toxic effects of composts with different initial antibiotic content. The germination index decreased with increasing dose of OTC in the manure. The difference of GI from the control compost without OTC was on average 76.8%.

On the 90th day it was found that all the composts from the contaminated manure had equally low toxicity levels as the control compost. The change of GI from the control compost without OTC was on average 94.7%. Interestingly different concentrations of oxytetracycline added to manure before composting did not cause significant differences to the level of phytotoxicity.

## DISCUSSION

Animal wastes from farms in raw form contain many toxic compounds that are harmful for plants [1]. Therefore, in the early stages of composting, compost is not commonly suitable for healthy plant growth [16]. The current study has showed results that are in close agreement with previous studies investigating the phytotoxicity of compost. Thus, in the initial stages (first 30 days) of composting of cow manure not containing antibiotics, high toxicity was observed, and toxicity was higher in the mixtures with a greater proportion of compost. By further observation of the composting process, the trend towards a reduction in the toxicity of the compost was revealed. It is known that in the process of composting, the original manures loses its harmful components, which is demonstrated by the fact that, when used as a fertilizer, such composts are able to create favorable conditions for plant growth [1]. In our study, toxicity continued to reduce up to the 90th day in the 1:1 mixture of compost and soil.

Manure from livestock contains a large quantity of antibiotics and there is a risk of their spread in the terrestrial and aquatic environments [4]. Composting is an effective way to reduce residues of antibiotics in animal waste from farms [17]. Many studies have demonstrated that manure loses its pathogenic properties, the many poisonous substances for plants, in the composting process and, consequently, it is effective in reducing the toxic effect on plant growth [6,10]. During the initial stages of composting, manure contaminated with OTC had the same high toxicity as those in the non-contaminated samples. However, the absence of additional OTC effect may be due to high toxicity of the compost mixtures themselves. On the 30th day of the composting process, there were significant differences in the level of toxicity between samples containing different concentrations of OTC. The association between increased toxicity and increased concentration of OTC in manure was noted. Therefore, the presence of antibiotics in the manure can affect the compost in a way which has increased the toxicity for plants. On 60th day the composts continued to have a toxic effect on oat plants. In addition, significant differences in the level of toxicity in composts containing different initial concentrations of OTC were found. It appears that, for this composting period (60 days), residual OTC concentrations can have a direct toxic effect on the growth and development of oat plants. Many studies also confirm the fact that when present in the composting process, antibiotics contained in the initial manure have a direct toxic effect on plants [18–20]. In addition, the presence of OTC in the manure could inhibit the microbial communities within the compost

that drive the composting process. Thus, inhibition of microbial communities could inhibit the composting process itself, and, consequently, create a natural decrease in phytotoxicity [17]. Many studies also provide conclusions explaining the indirect effect of antibiotics on the toxicity of composts due to the deceleration of the composting process caused by the inhibition of the functioning of microbial communities [1,9,17].

On the 90th day of the composting process, composts had low toxicity towards oat plants. At this stage of the composting process, there was no difference in the level of the toxicity of composts with different amounts of antibiotic. It is likely that the decrease in the toxicity at the end of the composting process is due to the gradual decomposition of OTC in composting mixtures. Our previous study found that the destruction of OTC when added to the soil occurs at a level of 98% after a 35 days incubation period [21]. Other works also indicate the gradual destruction of antibiotics in soil and manure [10,11,22].

## SUMMARY

In this work, an assessment of the change in phytotoxicity in the composting of manure, both pure and containing antibiotics was conducted. It showed a regular decrease in the toxicity of compost, from manure that does not contain antibiotics over the composting period. On the 60th day of composting, the germination index increased to 40.9%, 35.9% and 25.6% in the 1:1, 2:1 and 3:1 compost and soil mixtures, respectively. On the 90th day in the 1:1 mixture of compost and soil, the germination index was 106.6%, which is 2.6 times higher than in the same mixture the 60th day. By the end of composting (90 days), composting mixtures containing manure contaminated with oxytetracycline, also showed a reduction in toxicity. The lowest levels of phytotoxicity at the end of composting were found in soil and compost mixtures in a 1:1 ratio regardless of the presence of the antibiotic in the original manure.

## CONCLUSION

The results of this study showed that in the early stages of composting, composts, from both pure manure and from those contaminated with antibiotic had a significant level of phytotoxicity. The most rapid decrease in phytotoxicity occurred in compost prepared with pure manure. At the end of composting, the different initial concentrations of oxytetracycline in the manure did not cause significant differences in the level of phytotoxicity. However, in the process of composting (day 60), initial antibiotic concentration did play a role in the compost toxicity.

### CONFLICT OF INTEREST

There is no conflict of interest.

### ACKNOWLEDGEMENTS

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### FINANCIAL DISCLOSURE

None

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