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INFLUENCE OF TETRACYCLINE ON MICRONUTRIENTS DISTRIBUTION IN BEAN PLANTS

Mariana-Nicoleta BADEA[1], Ana Maria ANGHEL[2], Alexandru IVANOV[3], Violeta-Monica RADU[4]

Use of antibiotics in agriculture may cause serious problems, because their presence generates antibiotic resistant bacteria that can reach the human body through various ways – directly, by ingestion of food or indirectly, through environmental contamination. Because about 75% of the ingested antibiotics pass unaltered through the digestive tract, the animal manure contains both antibiotics and antibiotic resistant bacteria. In most cases, these wastes are stored in manure pits or scattered on open field, leading to the soil contamination, as well as to the interaction with soil components. Among antibiotics, tetracycline presents a special interest because it has the ability to form complexes with metals, including micronutrients. This paper presents the influence of tetracycline on micronutrients uptake capacity by bean plants grown on soil contaminated with tetracycline in different concentrations.

Keyword: antibiotic pollution, tetracycline, contamination, micronutrients

1. Introduction

Since their discovery, antibiotics have been used in infectious diseases, previously lethal for humans and animals. The most antibiotics are used to treat infections in humans and animals, but important quantities of them are also used as growth promoters at sub-therapeutic levels. Approximately half of all antibiotics produced in the world were destined for use in food animals [1].

Land spreading of manure containing antibiotics appears to be the dominating pathway for the release of antibiotics in the terrestrial environment, respectively in plants.

The issue of antibiotics residues in meat, honey and milk was studied for many years, but limited data are available regarding the impact of antibiotics on

[1] Senior researcher, The National Institute for Research and Development in Environmental Protection (INCDPM), Bucharest, Romania, e-mail: maragara2000@yahoo.com
[2] Senior researcher, The National Institute for Research and Development in Environmental Protection (INCDPM), Bucharest, Romania, e-mail: ana6209@yahoo.com
[3] Junior researcher, The National Institute for Research and Development in Environmental Protection (INCDPM), Bucharest, Romania, e-mail: aa_ivanov.incdpm@yahoo.com
[4] Senior researcher, The National Institute for Research and Development in Environmental Protection (INCDPM), Bucharest, Romania, e-mail: radumonica33@yahoo.com

plants grown on soil fertilized with manure containing antibiotics. In 2005, a study performed on green onion, cabbage, and corn found that plants can take up small amounts (2 to 17 ng/g of fresh tissue) of chlortetracycline from soil which had been amended with swine manure known to contain that antimicrobial [2].

Tetracyclines are the most common antibiotics present in manure, with concentrations ranging between 23 and 46 mg/kg in pig manure [3].

The fate of tetracycline in soil depends on pH and some metals, including few micronutrients, because tetracycline forms complexes, due to its B- and C-ring oxygen atoms. The complexes are easily adsorbed on soil [4], influencing the growth and development of plants.

The micronutrients are chemicals found in plant tissues in very small quantities (oligoelements), being components of enzymes, vitamins and hormones and they contribute to plant metabolism, increase the enzyme activity, stimulate biochemical reactions (synthesis of starch, sugar, proteins, lipids, nucleic acid, etc.).

There are 7 micronutrients widely recognized: boron (B), chlorine (Cl), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), and zinc (Zn). Other authors consider cobalt (Co) [5] and nickel (Ni) [6] as micronutrients, too.

The growth and development of higher plants are strongly influenced by boron, molybdenum and zinc. Boron is important for cell wall and membrane synthesis and structure [7].

Cobalt is important for leguminous plants that need it for nitrogen fixing bacteria [5].

Manganese is essential for chlorophyll in the plant and copper is involved in protein synthesis [8].

Iron has an important role in chlorophyll synthesis (even it is not part of the chlorophyll) and is involved in: nitrogen fixation, photosynthesis electron transfer, cytochrome enzyme systems and others [9].

Manganese activates an enzyme involved in photosynthesis. It is also a component of several enzyme systems and a structural component of metalloproteins and takes part in redox reactions.

Nickel seems to be involved in urea conversion to ammonia.

The micronutrient levels cover a large range of concentrations, from less than 1 ppm (Mo) to more than 100 ppm (Fe) and hundreds or thousands ppm (Cl) [10].

Each metal has the chemical properties to form complexes and up to 40% of proteins are bound with a metal [11].

This paper presents the impact of tetracycline on the micronutrients uptake on different parts of bean plants *(Phaseolus vulgaris)*, cultivated on soil contaminated with different tetracycline concentrations.

2. Experimental

Materials

The bean seeds and the soil used for this study were provided by the market. The seeds were of the type intended for consumption (not for seeding). Tetracycline, intended for human use, was purchased from the drugstore (caplets containing 250 mg tetracycline). Metal standard solutions (1000 mg/L in HNO_3) were purchased from Merck.

Plant growth test

The present experiment was conducted from the end of February until middle May 2014, over 80 days period of time. The seeds were thoroughly rinsed with bi-distilled water in order to remove any potential contaminant that could influence the experiment.

The germination rate was evaluated to be 85%. Six sets of 3 pots each were filled with 1000 g of garden soil. The soil from five sets was spiked with 50, 100, 200, 300 and 500 μg/Kg tetracycline. The tetracycline solution of 1 mg/L was prepared by dissolving the containment of a tetracycline caplet in a 250 ml flask in bi-distilatted water; subsequently, a solution of 100μg/L was obtained by dissolving 10 ml of 1mg/L tetracycline solution in a 100 mL flask. 0.5, 1, 2, 3 and 5 ml of this solution were added in pots in the dark, and the soil was mixed in a shaker for 1 h) and subsequently 10 bean seeds were cultivated in every pot at a depth of 1-2 cm. The sixth set of pots were not spiked with tetracycline solution and represents the control set.

The pots were watered with bi-distilled water in order to eliminate any interfering compound possibly present in the tap water, preventing the water leaching. The plants were kept in a room at 18-30 °C temperature range, in natural light corresponding to the test period.

Extraction and analysis

The stems, leaves and pods of every plant were harvested 80 days after sowing. The plants parts were washed with tape water, followed by distilled water and oven dried at 80 ºC temperatures until the constant mass was obtained.

The dried samples were smashed and powdered and 0.1 – 0.2 grams of each powdered sample was digested for metallic micronutrients: Fe, Mn, Co, Zn, Cr, Ni and Cu.

A blank-sample was also run. The experiment was performed in a clean environment.

Analytical Method

The micronutrients Cu, Co, Ni, Zn, Fe and Mn were determined by flame atomic absorption spectrometry using a spectrometer VARIAN SpectrAA – 250

Plus equipped with hollow cathode lamps. Peak height absorbance measurements were used as analytical signal. The spectrometer parameters were set at the recommended ones by user guide for metal analysis.

The results are expressed as mg micronutrient/kg dried weight.

3. Results and discussions

Bean plants have sprung up to 5-6 days after sowing and developed almost equally in all pots, excepting the pot containing 200 μg/kg tetracycline.

Table 1

Plant growth test results

No.	**Tetracycline concentration in soil (μg/kg)**	**Number of plants**	**Number of pods**
1	0 - control	8	6
2	50	10	4
3	100	9	10
4	200	2	2
5	300	9	6
6	500	8	9

The relatively small number of pods can be explained by the fact that the experiment has been performed in laboratory conditions that did not simulate the natural conditions corresponding to the real vegetation period (middle April – end of July or early August), without sufficient humidity in air, especially in very warm days. All plants have reached maturity after 80 days after sowing when they were harvested.

After samples mineralization and analysis, the following results have been obtained:

Table 2

Micronutrients concentration in different part of plants as function of contamination with tetracycline

Sample	TC mg/kg	Ni mg/kg	Cu mg/kg	Co mg/kg	Fe mg/kg	Mn mg/kg	Zn mg/kg
Leaves	0.000	12.339	12.506	1.020	184.324	49.468	63.670
Leaves	0.050	6.226	14.677	0.298	164.489	50.000	75.760
Leaves	0.100	8.497	21.187	0.841	851.081	77.962	64.852
Leaves	0.200	14.281	8.123	2.430	439.348	47.137	57.557
Leaves	0.300	0.000	0.000	0.479	18.826	0.160	0.000
Leaves	0.500	5.279	13.737	0.990	664.067	74.586	55.269
Stems	0.000	13.651	7.671	0.941	113.424	12.238	93.323
Stems	0.050	8.579	10.269	0.820	267.523	12.750	61.129
Stems	0.100	6.051	5.181	1.030	106.331	10.672	47.089
Stems	0.200	12.508	13.247	0.466	97.640	16.378	89.547
Stems	0.300	3.154	4.045	0.360	62.795	5.787	40.449
Stems	0.500	9.530	8.961	0.430	94.925	9.261	53.147
Pods	0.000	9.189	5.166	1.352	119.383	23.994	55.340

Pods	0.050	13.590	12.351	2.273	147.085	28.306	62.098
Pods	0.100	11.562	12.713	0.541	123.934	23.504	67.407
Pods	0.200	12.845	13.394	1.184	255.067	29.084	52.344
Pods	0.300	8.537	9.586	0.330	103.619	23.661	61.335
Pods	0.500	10.008	11.168	0.570	86.283	16.867	53.989

Animal drugs may be administered either by injecting them directly or by mixing them into feed and water. Studies on the metabolism of antibiotics in animals have shown that most antibiotics fed to animals are poorly absorbed in the animal gut, so that 90% of some antibiotics may be excreted as the parent compound [12]. These chemicals are excreted in urine and faeces, which in turn end up in manure and once in the environment, they can disrupt biogeochemical cycles or become pollutants. That's why the antibiotics resistance that can result from the wide-scale use of antibacterial in animals became a real problem. The fate of antibiotics in soils depends on sorption and desorption processes and leaching, that are strongly influenced by the reactions between the antibiotics and the soil. The antibiotics concentration in slurry manure ranges from trace to greater than 216 mg/L [13]. The antibiotics remain stable during manure storage and following the manure applications on agricultural fields the antibiotic residues reach in soil [14]. There are no guidelines regarding the presence of contaminants such as hormones and antibiotics in manure [15], even the organic farming supposes the use of manure instead of synthetic fertilizers.

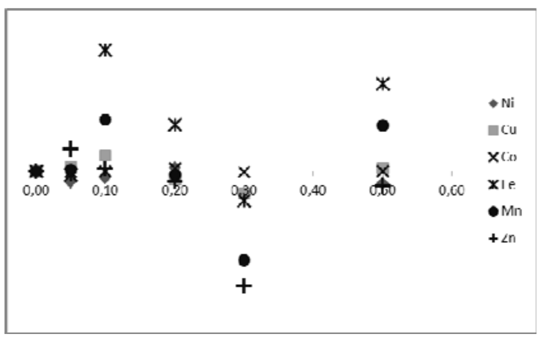
Among the antibiotics present in the environment, tetracycline is frequently detected. In the samples collected from soils fertilized with manure, concentrations of 86.2 (0−10 cm), 198.7 (10−20 cm), and 171.7 μg/kg (20−30 cm) tetracycline and 4.6−7.3 μg/kg chlortetracycline were found. The higher content of tetracycline was explained by repeated fertilizations with liquid manure that release antibiotic in environment, build up persistent residues and accumulate in soil [16]. The residual tetracycline forms complexes with the metallic micronutrient, modifying their natural uptake.

The present experiment showed that the micronutrients uptake is different, depending on the concentrations of Tetracycline in soil and part of plant.

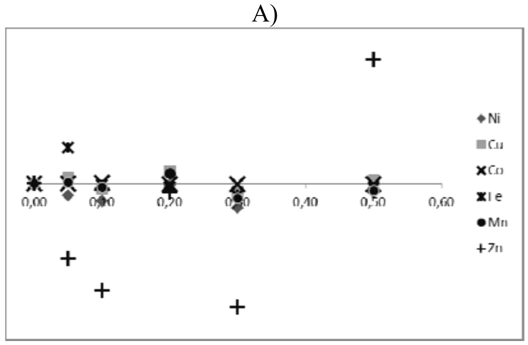
The greatest uptake, comparing with the control was noticed in leaves (Fig.1 A) for all micronutrients in the 0.05-0.2 mg/kg tetracycline concentration range, with a maximum for all micronutrients at 0.1 mg/kg tetracycline in soil, excepting Ni and Co. The smallest micronutrients concentration was noticed at 0.3 mg/kg tetracycline in soil for all elements, excepting Ni, Cr and Co, followed by an increase of all micronutrients concentrations at 0.5 mg/kg tetracycline in soil. Regarding the stems, the micronutrients concentration were likely for all tetracycline concentrations, excepting Zn, that had a decrease in 0-0.3 mg/kg range, followed by a significant increase to 0.5 mg/kg tetracycline in soil.

The behavior of micronutrients in pods was similar with the behavior in leaves, with a maximum uptake at 0.2 mg/kg tetracycline in soil, excepting Zn, whose concentration decreases comparing with the control. The copper uptake was greater then the control in all parts of plants for the most tetracycline concentrations - excepting that for leaves at 0.2 and 0.3 mg/kg tetracycline and for stems at 0.1 and 0.3 mg/kg tetracycline. Ni accumulated more than control in leaves and pods, Co in leaves, stems and pods at different tetracycline concentrations, Fe in leaves and pods with greater uptake in leaves at 0.1 mg/kg tetracycline in soil, Mn in all plant's part at 0.05 mg/kg tetracycline in soil and Zn in leaves and pods for the most tetracycline concentrations.

The contamination with 0.05 mg/kg tetracycline in soil determines a greater uptake of all micronutrients in pods. This concentration corresponds to 100% germination capacity, but the productivity (number of pods/plant) is very low (0.4 pods/plant).



A)



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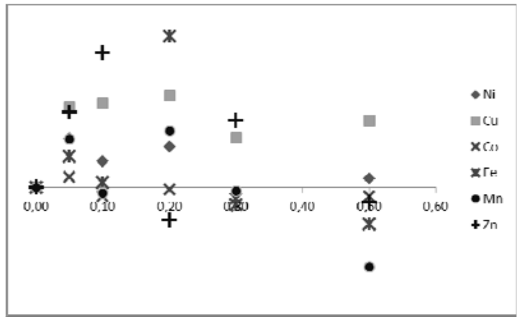

C)

Fig. 1. Micronutrients concentration in different part of plants comparing with the control sample: A) leaves; B) stems; C) pods

The best productivity (1.11 and 1.25 pods/plant) was noticed for 0.100 and 0.500 mg/kg tetracycline in soil, while the productivity for the control sample was 0.75pods/plant. This productivity is correlated with higher Cu and lower Co contents in pods (2 times than the control sample) and very high Fe level in leaves, while others' micronutrients concentration is similar to that of the control sample.

4. Conclusions

The organic agriculture uses manure as fertilizers instead of synthetic ones. Because the antibiotics pass unaltered through the digestive tract the animal manure may content different antibiotics, especially when the manure is not sufficiently maturated. Tetracycline is one the most common antibiotic that can be found in manure, even after more than 100 days, and subsequently may reach in soil and plants. This scenario is very possible because the European regulations do not impose the control of the antibiotics concentration in manure prior disposal on the field. Tetracycline interacts with soil component, especially with metals, generating complexes that are differently metabolized by plants, so the distribution of the micronutrients in plants differs comparing with the control.

Tetracycline contamination determines a higher uptake of micronutrients in leaves and pods for lower levels of tetracycline in soil, while the uptake of micronutrients in stems is similar to the control sample. The productivity of bean plants seems to be influenced by micronutrients interaction with tetracycline in soil. A tetracycline level in soil corresponding to 0.100 mg/kg seems to enhance the proteins synthesis in pods (higher Cu levels in pods) and chlorophyll synthesis (high levels of Fe in leaves). The contamination with very low tetracycline levels

(0.050 mg/kg in soil) seems to have negative effects on productivity, generating a higher uptake of all micronutrients in pods.

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