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REMOVAL-DEGRADATION COW'S WASTEWATER AND ANTIBIOTIC OF ERYTHROMYCIN FROM AQUEOUS SOLUTIONS BY PHYTOREMEDIATION

Stavros Georgopoulos
PhD Student, Department of Environmental Engineering, School of Engineering, University of Patras, 2 Georgiou Seferi St. Agrinio, Greece

ABSTRACT

For at least 300 years, the ability of plants to remove contaminants from the environment has been recognized and taken advantage of in applications such as land farming of waste. In more recent years, as recognition grew of the damage resulting around the world from decades of an industrial economy and extensive use of chemicals, so did interest in finding technologies that could address the residual contamination, which one of these technologies is the above method; phytoremediation. This work presents preliminary findings on the removal of toxicity of a cow-farm wastewater and the removal of the antibiotic of erythromycin. For this reason the search was focused on the use of plants and more specifically in plant stems. This method has low cost and is effective in the removal or degradation of those compounds. However, further research is necessary to establish a unified method (including biological treatment methods) which will be practically enabled to complete the removal or the degradation of those compounds from waters or soil.

Keywords: *clean up technology, phytoremediation, removal degradation, cow's wastewater, erythromycin*.

I. INTRODUCTION

The earth has a large amount of water which covers the 70,9% of its surface and composes the most necessary component of life (UN, 2014). Nearly the 96,5% of the water that exists on the earth is salt water while the 2,47% is stored in icebergs, in a solid form, in underground carrying water which is not accessible to humans or there is as a soil moisture. So, the amount of water that is available for human uses is 0,3% [Gleick, (1993), p.13].

The depletion of many natural sources of water as well as the pollution introduced by wastes that humans are responsible for, increase the problem of clean fresh water which is available on the earth. It is estimated that more than 40% of the world population is affected by the lack of the water because of political, economical and climatic conditions. Furthermore, more than 25% of the world population suffers from health problems which are related with the lack of pure water. According to United Nations data, more than 1 billion inhabitants of the earth have not access on pure drinking water. Moreover, the use of water by 2027 will be increased 40% and 17% more water will be necessary for the increasing population of the earth (Koch, 1993, p.305; Molina, 2007; Rajan et al., 2018; Tarrass and Benjelloun, 2012; Verstraete et al., 2009).

Large quantities of industrial, agricultural and local sources are ended on surface waters such as rivers, lakes and sea. Water pollution has obviously negative results to the organisms of the aquatic ecosystems. Furthermore, it constitutes a significant danger for the human health (Ohe et al., 2004; Rajan et al., 2018; Zhang et al., 2018). The surface waters, which contain a lot of unknown components, are used as a source of drinking water and irrigation. Therefore, the pollution of these waters is developed to an important problem in more and more areas (Arusievicz Nunes et al., 2011).

For at least 300 years, the ability of plants to remove contaminants from the environment has been recognized and taken advantage of in applications such as land farming of waste. Over time, this use of plants has evolved to the construction of treatment wetlands or even the planting of trees to counteract air pollution. In more recent years, as recognition grew of the damage resulting around the world from decades of an industrial economy and extensive use

of chemicals, so did interest in finding technologies that could address the residual contamination, among them phytoremediation (EPA, 2000, p.6; Mleczek et al., 2019).

Phytoremediation is a group of technologies which use plants (and their possible correlation with microorganisms) for the in situ partial or full restoration of contaminated soils, sludges, sediments and surface or groundwater. Phytoremediation technologies are used for dealing with both organic and inorganic pollutants. These pollutants include hydrocarbons, chlorinated compounds, pesticides, antibiotics, explosives, heavy metals, metalloids and radioactive materials (Alkorta and Garbisu, 2001; Atugoda, 2018; Ensley, 2000, p.3-11; Kosiorek and Wyszkowski, 2019).

Phytoremediation, in addition to remove pollutants, is also capable to metabolize organic compounds to inorganic or degrade these to non or less toxic compounds. This process can be achieved in plant tissues (Dietz and Schnoor, 2001; Meagher, 2000; Salt et al., 1998).

After the process of phytoremediation, the plants harvested will potentially have the pollutants accumulated in their tissues. Depending on the type of contamination, the plants can either be disposed of or used in alternative processes, such as burning for energy production. In essence, phytoextraction (one method of phytoremediation) removes pollutants from contaminated soils, concentrates them in biomass and further concentrates the pollutants by combustion (Dipu, 2011; Peuke and Rennenberg, 2005).

In order to evaluate the success of a pollutant removal method, toxicity of the treated water has to be assessed. There are many bioassays to assess the above mentioned toxicity (Farre and Barcelo, 2003; Tothill and Turner, 1996). One of these can be achieved by plants (Farre and Barcelo, 2003). Generally, the plant bioindicator offers advantages such as a wide range of final assessment (percentage of vegetation, percentage of root growth, weight of biomass and enzyme activity), low cost of support and quick start of bioassay with particular advantages for the dynamic ecotoxic review of the wastewater. For this purpose they are used species such as oats (*Avena sativa* L.), chinese cabbage (*Brassica campestris* L.) and onion (*Allium cepa* L.) (Ferrari et al., 1999; Fiskesjo, 1993).

The method of *Allium cepa* L., which is a qualitatively method, is an easy and sensitive tool for the measurement of the total toxicity caused by chemicals expressed by the root growth inhibition of onion bulbs. The degree of toxicity of the test chemicals is estimated by measuring the length of the roots in the third day of the experiment (Fiskesjo, 1993).

The target of this work is to remove toxicity of a cow-farm wastewater and the removal of a very persistent antibiotic, i.e. erythromycin which is extensively used in animals, with the method of phytoremediation. Erythromycin ($C_{37}H_{67}NO_{13}$) has been encountered in the surface water in a number of places (Christian et al., 2003; Ferrer and Thurman, 2012; Roberts and Thomas, 2006).

II. METHOD & MATERIAL

For the purpose of remediation, stems of plants were collected in two different periods.

a. Cow-farm wastewater

Especially, for the treatment of cow's wastewater, stems of a number of local plants with a length of about 30-35 cm and 1 cm diameter were collected from the west and east side of Lake Stratos in the region of Agrinio (38 37'00" N 21 24"00E/38.6167 N) of the Prefecture of Aetoloacarnania in west Greece on the 06/04/2013. Their identified as in genus based on keys. More specifically, the flores Hellenica (Strid and Tan, 1986; 1991; 1997; 2002) Italia (Pignatti, 1941a; 1941b; 1982) and Europe (Tutin, 1964; 1968; 1972; 1976; 1980) were used. Furthermore, the e-database of "Euro+Med PlantBase" (http://ww2.bgbm.org/EuroPlusMed/query.asp\) was used.

Then, the stems (with the leafs) were placed in plastic water bottles filled with 0,5 L of wastewater containing 342,86 mL of tap water and 57,14 mL of filtered wastewater from a cow-farm. In addition, the solution that was placed in plastic bottles containing the nutrient elements shown in Table 1 (Fiskesjo, 1993).

Table 1. Nutrients used for the plants employed in the phytoremediation process (cow-farm wastewater)

Compound	Concentration [g L^{-1}]
$Ca(NO_3)_2 \cdot 4H_2O$	0,177
KNO_3	0,152
$MgSO_4 \cdot 7H_2O$	0,185
KH_2PO_4	0,101
EDTA	0,165
Trace elements:	
$MnSO_4 \cdot H_2O$	4,122 $*10^{-6}$
$CuCl_2 \cdot 2H_2O$	0,484 $*10^{-6}$
$NaMoO_4 \cdot 2H_2O$	0,011 $*10^{-6}$
$ZnSO_4 \cdot 7H_2O$	0,009 $*10^{-6}$
H_3BO_3	1,715 $*10^{-6}$

The top of each bottle was covered with a transparent membrane so as to minimize water losses due to evaporation. The plants were left indoors at room temperature under natural sunlight but not in direct contact with the sun for just over fourteen days.

On 20/04/2013 samples were extracted from each bottle to assess the toxicity of cow-farm wastewater using the onion *Allium cepa* L. method. More specifically, Dutch commercial onions with a diameter nearly 1,0 to 1,5 cm and a length of roots zero were used. The experimental procedure took place at room temperature where the onions did not come into direct contact with the sun. Thirty six onions were used in total six of which were used as reference. Three of the reference onions were placed in enriched with nutrients tap water. The nutrients kind and concentrations employed in the reference onions are presented in Table 2 (Fiskesjo, 1993).

Table 2. Nutrients employed in onions for the toxicity study (cow-farm wastewater)

Compound	Concentration [g L^{-1}]
$Ca(NO_3)_2 \cdot 4H_2O$	0,0236
KNO_3	0,0202
$MgSO_4 \cdot 7H_2O$	0,0246
KH_2PO_4	0,0135
EDTA	0,0220
Ιχνοστοιχεία:	
$MnSO_4 \cdot H_2O$	0,550 $*10^{-6}$
$CuCl_2 \cdot 2H_2O$	0,065 $*10^{-6}$
$NaMoO_4 \cdot 2H_2O$	0,001 $*10^{-6}$
$ZnSO_4 \cdot 7H_2O$	0,001 $*10^{-6}$
H_3BO_3	0,229 $*10^{-6}$

The remaining three reference onions (indicated hereinafter as Reference*) were included the ratio of tap water, of filtered wastewater and the nutrient elements that they were placed in plastic bottles. The amount of nutrient elements employed was reduced by 14,3% to counterbalance the amount of nutrient elements absorbed by the stems of the plants used for phytoremediation until the day the toxicity tests were conducted.

The remaining onions were placed in the treated solutions containing cow-farm wastewater. Three samples were used per plant species. All the samples contained 11 mL of solution and they were placed in glass test-tubes (12 mL). The onions were placed on the top of each tube which about 1/3 of them was inside of the respective tube. During the three days of the testing, the solution that was absorbed by each onion (0,5 - 1 mL) was replaced it every 8 hours in order to maintain the height of the solution in the desired level.

In the third day of the experiment, the length of each root had been measured by ruler (Peliran). The average length of the roots per onion as well as the average length of the roots per plant species/reference/reference* was calculated.

b. Removal of erythromycin

A similar process was followed for the removal of erythromycin from surrogate wastewater. Water-soluble erythromycin 5% was used (Erythromycin thiocyanate 5 g, Secondary compounds 100 g) in powder which is supplied with drinking water to the birds. Two different sets of experiments were conducted concerning this antibiotic. On the 26/12/2013 stems of a number of plants with a length of 40 cm for the first experiment and 10 cm for the second experiment, respectively, were collected from the extensive area of Nea Zouzouli, Kastoria, North-Western Greece. Their identified as in genus based on keys. More specifically, the flores Hellenica (Strid and Tan, 1986; 1991; 1997; 2002) Italia (Pignatti, 1941a; 1941b; 1982) and Europe (Tutin, 1964; 1968; 1972; 1976; 1980) were used. Furthermore, the e-database of "Euro+Med PlantBase" (http://ww2.bgbm.org/EuroPlusMed/query.asp\) was used. Three different stems were collected for each genus and each experiment.

They left in the dark, at room temperature, with the roots and the lower stem submerged in tap water all the night so that attached soil came loose. The next day they were cleaned very well to remove any remaining mud. To those that were not cleaned very well, the roots as well as 2-4 cm (for the first experiment) and 1,5-2 cm (for the second experiment) of the lower part of the stem had been cut it before the each experiment started. Table 3 (for the first experiment) and Table 4 (for the second experiment) represent which species were submerged with roots and which without roots.

Table 3. Indicate which species were submerged with roots (R) and which without roots (NR), for the first experiment (antibiotic of erythromycin)

Broccoli (*Brassica* sp.)	NR	Cabbage (*Brassica* sp.)	NR
Cauliflower (*Brassica* sp.)	NR	Pine wood (*Pinus* sp.)	R
Bramble (*Rubus* sp.)	R		

Table 4. Indicate which species were submerged with roots (R) and which without roots (NR), for the second experiment (antibiotic of erythromycin)

Leek (*Allium* sp.)	R	Strawberry (*Fragaria* sp.)	NR
Nettle (*Urtica* sp.)	NR	Grass (*Agrostis* sp.)	R
Basil (*Ocimum* sp.)	NR	Lettuce (*Lactuca* sp.)	R
Chicory (*Cichorium* sp.)	NR		

Subsequently, the stems employed in the first experiment with their leaves in the top of each stem, were placed in 0,5 L capacity glass bottles containing 400 mL of solution. One of the three stems of each species was used as a reference and it was submerged to pure tap water whereas the remaining two stems were submerged in a tap water containing erythromycin in a concentration of 1 g L^{-1}. The two stems employed in the second experiment, also with their leaves on the top of each stem, were placed in glass tubes of 12 mL containing 10 mL of the aqueous solution containing erythromycin in the same concentration. The third stem was used as a reference with their leaves on the top of each stem and it was submerged in a 10 mL tap water.

The top of each bottle/tube was covered with transparent membrane so as to minimize water losses due to evaporation. The plants were left under natural sunlight but not in direct contact with the sun for just over seventeen days for the first experiment and fifteen days for the second experiment.

On the 17th day and 15th day, respectively, the plants were removed from the solutions. The height of each stem was measured by ruler (Peliran) and titrate the final volume of the solutions. Furthermore, samples were extracted from each bottle/tube and they were filtered with a TF Whatman, 0,45 μm, 25 mm filter for removing any particulate matter and the contained total organic carbon (TOC) in each solution was measured. The TOC measurement was

carried out by a TOC-$V_{CSH/CSN}$, TOC Analyzer, Schimadzu ® equipped with a NDIR detector. The instrument calibration was performed using potassium hydrogen phthalate standard solutions for total carbon (TC) calibration, while for inorganic carbon (IC) sodium hydrogen carbonate and sodium carbonate solutions were prepared.

III. RESULT AND DICUSSION

As it is referred above, the removal or degradation of cow-farm wastewater was determined qualitatively measuring the length of the roots. The following Table represents the stems of plants that were collected for the purpose of remediation. In addition, the percentage of growth of the average length of the roots per plant species/reference* based to the average length of the roots of the reference.

Table 5. The stems of plants that were collected for the purpose of remediation. The percentage of growth of the average length of the roots per plant species/reference* based to the average length of the roots of the reference (cow-farm wastewater)

Samples	% of reference	Samples	% of reference
Reference	100	Spruce (*Abies* sp.)	34,31
Reference*	34,14	Willow (*Salix* sp.)	41,16
Wild pear tree (*Pyrus* sp.)	44,66	Coffee tree (*Coffea* sp.)	42,91
Pear tree (*Pyrus* sp.)	28,87	Wild fig tree (*Opuntia* sp.)	44,66
Bramble (*Rubus* sp.)	18,35	Nettle (*Urtica* sp.)	25,01
Thistle (*Silybum* sp.)	23,61	Phillyrea (*Phillyrea* sp.)	11,68

It is worth mentioned that the standard deviation and the standard error are range in the normal level. Especially, the standard deviation is range from 0,03 to 0,13 cm and the standard error is range from 0,02 to 0,08 cm while the length of the roots are range from 0,1 to 1,4 cm.

As can be seen, the average length of the roots of the reference* are too smaller than reference. This means that the aforementioned wastewater are enough toxic. Moreover, from the above Table, it is comprehensible that many stems removed or degraded the cow's wastewater (Wild pear tree, Spruce, Willow, Coffee tree, Wild fig tree). However, the average length of the roots of some plant species is smaller than the reference* (Pear tree, Bramble, Thistle, Nettle, Phillyrea). That occurs because during the experimental procedure products were formed that were more toxic than the initial concentration.

Furthermore, reduction of toxicity of cow's farm wastewater is achieved by different species of plant (Ding, 2015; Gottschall et al., 2007; Rajan et al., 2018). The phytoremediation took place in a constructed wetland or in artificial diluted solutions with microalgae or *Typha* species. In that cases the mainly parameters that examined are the COD, the BOD, the removal of Phosphorus and Nitrogen where the reduction of the above mentioned parameters is observed.

Concerning the antibiotic of erythromycin, Table 6 (for the first experiment) and Table 7 (for the second experiment) presents the stems of plants that were collected for the purpose of remediation. In addition, they represent the initial and final volume of each solution as well as the initial and final height of each stem.

Table 6. The stems of plants that were collected for the purpose of remediation, the initial and final volume of each solution as well as the initial and final height of each stem (antibiotic of erythromycin-first experiment)

Stems of plant taxa	Initial volume of solution (mL) (27/12/2013)	Final volume of solution (mL) (13/01/2014)	Initial height of stems of plant taxa (cm) (27/12/2013)	Final height of stems of plant taxa (cm) (13/01/2014)
Broccoli I (*Brassica* sp. I)	400	376	24	36
Broccoli II (*Brassica* sp. II)	400	395,8	24	27
Broccoli Reference (*Brassica* sp. Reference)	400	382	24	28
Cauliflower I (*Brassica* sp. I)	400	393,3	14	14
Cauliflower II (*Brassica* sp. II)	400	398,1	14	15
Cauliflower Reference (*Brassica* sp. Reference)	400	395,4	14	17
*Bramble I (*Rubus* sp. I)	400	386	40	45
*Bramble II (*Rubus* sp. II)	400	384,7	40	42
*Bramble Reference (*Rubus* sp. Reference)	400	381,7	40	43
Cabbage I (*Brassica* sp. I)	400	395,4	12	22
Cabbage II (*Brassica* sp. II)	400	386,8	12	22
Stems of plant taxa	Initial volume of solution (mL) (27/12/2013)	Final volume of solution (mL) (13/01/2014)	Initial height of stems of plant taxa (cm) (27/12/2013)	Final height of stems of plant taxa (cm) (13/01/2014)
Cabbage Reference (*Brassica* sp. Reference)	400	384	12	14
*Pine wood I (*Pinus* sp. I)	400	339,1	40	45
*Pine wood II (*Pinus* sp. II)	400	273,2	40	56
*Pine wood Reference (*Pinus* sp. Reference)	400	324	40	45

*Each of the three stems of Bramble and Pine wood were collected from the same tree and from the same height (~1m).

Table 7. The stems of plants that were collected for the purpose of remediation, the initial and final volume of each solution as well as the initial and final height of each stem (antibiotic of erythromycin-second experiment)

Stems of plant taxa	Initial volume of solution (mL) (27/12/2013)	Final volume of solution (mL) (11/01/2014)	Initial height of stems of plant taxa (cm) (27/12/2013)	Final height of stems of plant taxa (cm) (11/01/2014)
Leek I (*Allium* sp. I)	10	2,14	10	12
Leek II (*Allium* sp. II)	10	3,11	10	17
Leek Reference (*Allium* sp. Reference)	10	0,5	10	13
Nettle I (*Urtica* sp. I)	10	4,17*	14	15*

Nettle II (*Urtica* sp. II)	10	3,315*	14	15*
Nettle Reference (*Urtica* sp. Reference)	10	0,315*	14	14*
Basil I (*Ocimum* sp. I)	10	3,3	10	13

Table 7 (Continue): The stems of plants that were collected for the purpose of remediation, the initial and final volume of each solution as well as the initial and final height of each stem (antibiotic of erythromycin-second experiment)

Stems of plant taxa	Initial volume of solution (mL) (27/12/2013)	Final volume of solution (mL) (11/01/2014)	Initial height of stems of plant taxa (cm) (27/12/2013)	Final height of stems of plant taxa (cm) (11/01/2014)
Basil II (*Ocimum* sp. II)	10	4,35	10	10
Basil Reference (*Ocimum* sp. Reference)	10	4,505	10	10
Chicory I (*Taraxacum* sp. I)	10	4	6	8
Chicory II (*Taraxacum* sp. II)	10	0,83	6	14
Chicory Reference (*Taraxacum* sp. Reference)	10	2,01	6	7
Strawberry I (*Fragaria* sp. I)	10	0,735	6	7
Strawberry II (*Fragaria* sp. II)	10	1,51	6	10
Strawberry Reference (*Fragaria* sp. Reference)	10	1,605	6	7
Grass I (*Agrostis* sp. I)	10	6,38	14	15
Grass II (*Agrostis* sp. II)	10	6,35	14	16
Grass Reference (*Agrostis* sp. Reference)	10	7,53	14	14
Lettuce I (*Lactuca* sp. I)	10	4,44	10	11
Lettuce II (*Lactuca* sp. II)	10	4,2	10	10
Lettuce Reference (*Lactuca* sp. Reference)	10	4,51	10	11

*The measurement of the final height of the stems of Nettle and the titrate of respective solution were conducted on 03/01/2014.

From the Tables 6 and 7, general, it is observed that the results have repetition. Moreover, during the above mentioned experimental procedures, the three different stems for each genus absorbed similar amount of aqueous solution. The exceptions are the pine wood for the first experiment and the leek, the nettle and the chicory for the second experiment. More specifically, one of the stems of pine wood that submerged in aqueous solution containing erythromycin absorbed more amount of aqueous solution than the other two stems which submerged in tap water and in the aqueous solution of erythromycin, correspondingly. In relation to the second experiment, the reference of the stems of the leek and the nettle absorbed more amount of aqueous solution than the other two stems that submerged in the aqueous solution of erythromycin. Regard to the stems of chicory, a slight difference is perceived concerning the amount of aqueous solution that was absorbed by the respective stem.

From the Tables 6 and 7, it is comprehensibled that at the end of each experiment most of the stems were taller than they were at the beginning of the aforementioned experiments. However, some stems had the same height in the

beginning and at the end of each experiment. That stems from the first experiment are Cauliflower I while from the second experiment are Nettle Reference, Basil II, Basil Reference, Grass Reference and Lettuce II.

After 17 days for the first experiment and 15 days for the second experiment the TOC analysis (in mg L^{-1}) of the aqueous solutions was measured for each stem. Furthermore, the TOC of the initial concentration and of the tap water was measured. Table 8 for the first experiment and Table 9 for the second experiment displays these measurements.

Table 8. TOC analyses (in mg L^{-1}) of the initial concentration, of the tap water and of the aqueous solution for each stem (antibiotic of erythromycin-first experiment)

Samples that were submitted in TOC analysis	TOC
Initial concentration	408,4
Tap water	0,538
Broccoli I (*Brassica* sp. I)	386,1
Broccoli II (*Brassica* sp. II)	236,4
Broccoli Reference (*Brassica* sp. Reference)	116,2
Cauliflower I (*Brassica* sp. I)	436,95
Cauliflower II (*Brassica* sp. II)	532
Cauliflower Reference (*Brassica* sp. Reference)	88,05
Bramble I (*Rubus* sp. I)	402,4
Bramble II (*Rubus* sp. II)	400,25
Bramble Reference (*Rubus* sp. Reference)	421,15
Cabbage I (*Brassica* sp. I)	464,95
Cabbage II (*Brassica* sp. II)	311,3
Cabbage Reference (*Brassica* sp. Reference)	340,3
Pine wood I (*Pinus* sp. I)	335,1
Pine wood II (*Pinus* sp. II)	344,9
Pine wood Reference (*Pinus* sp. Reference)	83,4

Table 9. TOC analyses (in mg L^{-1}) of the initial concentration, of the tap water and of the aqueous solution for each stem (antibiotic of erythromycin-second experiment

Samples that were submitted in TOC analysis	TOC
Initial concentration	408,4
Tap water	0,538
Leek I (*Allium* sp. I)	166,85
Leek II (*Allium* sp. II)	60,65
Leek Reference (*Allium* sp. Reference)	ND
Nettle I (*Urtica* sp. I)	104,6
Nettle II (*Urtica* sp. II)	63,1
Nettle Reference (*Urtica* sp. Reference)	ND
Basil I (*Ocimum* sp. I)	101,35
Basil II (*Ocimum* sp. II)	107,75
Basil Reference (*Ocimum* sp. Reference)	18,62

Chicory I (*Taraxacum* sp. I)	45,26
Chicory II (*Taraxacum* sp. II)	ND
Chicory Reference (*Taraxacum* sp. Reference)	50,7
Strawberry I (*Fragaria* sp. I)	242,4
Strawberry II (*Fragaria* sp. II)	236,8
Strawberry Reference (*Fragaria* sp. Reference)	244
Grass I (*Agrostis* sp. I)	69,25
Grass II (*Agrostis* sp. II)	63,75
Grass Reference (*Agrostis* sp. Reference)	64

Table 9 (Continue): TOC analyses (in mg L^{-1}) of the initial concentration, of the tap water and of the aqueous solution for each stem (antibiotic of erythromycin-second experiment)

Samples that were submitted in TOC analysis	TOC
Lettuce I (*Lactuca* sp. I)	84,65
Lettuce II (*Lactuca* sp. II)	112,85
Lettuce Reference (*Lactuca* sp. Reference)	55,7

ND: Not Detected

The solubility of erythromycin is slightly (Suarez and Ellis, wd). This is proven from the Tables 8 and 9 since 1000 ppm (1 g L^{-1}) were placed in each solution while the initial concentration that recorded by the TOC Analyzer was 408,4 ppm. In addition, from the Tables 8 and 9 it is comprehensibled that increasing of TOC was happened in the solutions of the references compared to the TOC of tap water. This means that there were reactions between each solution of erythromycin with the respective stem, as a result these stems take out Total Organic Carbons to the respective solution. So, the reduction of TOC of those solutions is greater than it is referred to the above Tables. It is greater against TOC of each reference minus TOC of the tap water.

Based on the above, the results of the TOC analyses of the aqueous solutions are display in the Table 10 for the first experiment and in the Table11 for the second experiment.

Table 10. TOC analyses (in mg L^{-1}) of the initial concentration and of the aqueous solution for each stem based on the references (antibiotic of erythromycin-first experiment)

Samples that was submitted in TOC analysis	TOC
Initial concentration	408,4
Broccoli I (*Brassica* sp. I)	270,438
Broccoli II (*Brassica* sp. II)	120,738
Cauliflower I (*Brassica* sp. I)	349,438
Cauliflower II (*Brassica* sp. II)	444,488
Bramble I (*Rubus* sp. I)	0
Bramble II (*Rubus* sp. II)	0
Cabbage I (*Brassica* sp. I)	125,188
Cabbage II (*Brassica* sp. II)	0
Pine wood I (*Pinus* sp. I)	252,238
Pine wood II (*Pinus* sp. II)	262,038

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Table 11. TOC analyses (in mg L^{-1}) of the initial concentration and of the aqueous solution for each stem based on the references (antibiotic of erythromycin-second experiment)

Samples that was submitted in TOC analysis	TOC
Initial concentration	408,4
Leek I (*Allium* sp. I)	140,488
Leek II (*Allium* sp. II)	34,288
Nettle I (*Urtica* sp. I)	78,238
Nettle II (*Urtica* sp. II)	36,738
Basil I (*Ocimum* sp. I)	83,268
Basil II (*Ocimum* sp. II)	89,668
Chicory I (*Taraxacum* sp. I)	0
Chicory II (*Taraxacum* sp. II)	ND
Strawberry I (*Fragaria* sp. I)	0

Table 11 (Continue): TOC analyses (in mg L^{-1}) of the initial concentration and of the aqueous solution for each stem based on the references (antibiotic of erythromycin-second experiment)

Samples that was submitted in TOC analysis	TOC
Strawberry II (*Fragaria* sp. II)	0
Grass I (*Agrostis* sp. I)	5,788
Grass II (*Agrostis* sp. II)	0,288
Lettuce I (*Lactuca* sp. I)	29,488
Lettuce II (*Lactuca* sp. II)	57,688

ND: Not Detected

From the Tables 10 and 11, generally, it is realized that the stems were removed or degraded a large amount of erythromycin and the secondary compounds of it. Particularly in the first experiment, the greater removal or degradation was marked in the stems of Broccoli II and Cabbage I but the shorter in the Cauliflower (I and II). For the second experiment, the greater removal or degradation was marked in the stems of Leek II, Nettle II and Lettuce I but the shorter in the Leek I. It is worth to refer that (almost) all the quantity of the organic compounds were removed or degraded from the stems of Brambles and Cabbage II for the first experiment and from the Chicory I, Strawberries and Grasses for the second experiment. Furthermore, it is understandable that the TOC of Cauliflower II is greater than the initial concentration. This means that the stem did not remove or degrade erythromycin and the secondary compounds of it, but it took out TOC to the respective solution.

Similar work was conducted by Pierattini et al. (2016). In this work *Populus alba* Villafranca clone was examined and the concentrations of erythromycin were 0.01, 0.1 and 1 mg L^{-1}. After 3 and 28 days of experiment, three plants for each treatment were harvested, carefully rinsed with deionized water and separated into roots, stem, and leaves. For all treatments, erythromycin was absorbed by each plant and mainly concentrated in roots. In addition, the concentration of that antibiotic in stem and roots did not vary in time, while an increasing trend has been observed in leaves. Furthermore, it is observed that exposure more concentration of erythromycin, more absorption was achieved by the organs of *Populus alba* Villafranca clone. Moreover, the photochemical efficiency of photosystem II and the quenching analysis of chlorophyll fluorescence were examined. More specifically, the photochemical efficiency of photosystem II did not show a dose-dependant trend. Concerning the quenching analysis of chlorophyll fluorescence, low nonphotochemical quenching and high photochemical quenching for the first week of erythromycin exposure was observed, depending on leaves position along the stem.

IV. CONCLUSION

The earth has a large amount of water which covers the 70,9% of its surface and composes the most necessary component of life, but the amount of water that is available for human uses is 0,3%. In connection with the depletion of many natural sources of water as well as the pollution introduced by wastes that humans are responsible for, increase the problem of the clean fresh water on the earth.

Large quantities of industrial, agricultural and local sources are ended on surface waters such as rivers, lakes and sea. The surface waters, which contain a lot of unknown components, are used as a source of drinking water and irrigation. Therefore, the pollution of these waters is developed to an important problem in more and more areas. That is why the scientific community did interest in finding technologies that could address the residual contamination; one of these technologies is phytoremediation.

The target of this work is to remove toxicity of a cow-farm wastewater and the removal of a very persistent antibiotic, i.e. erythromycin, with the method of phytoremediation. For this reason, stems of plants were collected in two different periods. Afterwards, the stems were placed in bottles/tubes where containing the respective wastewater. Subsequently, the removal of toxicity of a cow-farm wastewater and the removal of the antibiotic of erythromycin were determined. Especially, the removal of the toxicity of the cows wastewater was estimated qualitatively using the commercial onions *Allium cepa* L. This method is an easy and sensitive tool for the measurement of the total toxicity caused by chemicals expressed by the root growth inhibition of onion bulbs. Concerning the antibiotic of erythromycin the removal or degradation of that was calculated by automatic total organic carbon analyzer.

The stems of Wild pear tree, Spruce, Willow, Coffee tree and Wild fig tree, it is realized that they removed or degraded a large amount of cow's wastewater. For the antibiotic of erythromycin the correspondingly stems, which were removed or degraded a large amount of that antibiotic, are Broccoli II, Cabbage I, Leek II, Nettle II and Lettuce I. It is worth mentioned that the stems of Brambles, Cabbage II, Chicory I and Strawberries were removed or degraded all the quantity of the antibiotic of erythromycin.

In this work, the fate of wastewater of cows and of erythromycin in the stems was not measured. However, the above results show that plants which removing or degrading large quantities of these wastewater have the potential of being used for cleaning of correlatively waters or soils, in particular the plants that were used in the second experiment of erythromycin, because the amount of aqueous solution of erythromycin that was absorbed by these stems was a great. On the contrary, plants exhibiting low removal or degradation or formed products that were more toxic than the initial compound, potential are inappropriate for use for this purpose.

On the other hand, if edible fruit (like strawberry) is exposed to the above wastewater, the plant will absorb these compounds. Whether these will be transferred to the fruits of the plant or not, depends on whether the plant possesses the capacity of metabolizing them. Further studies are required in order to identify the fate of those compounds in the plant and more precisely in order to quantify how much of the absorbed compounds are removed by transpiration, how much are metabolized by the plant and how fast these processes occur. The results of such studies will be enable for us to identify the appropriate plants for water and soil decontamination, but also fruit plants which can or should not be planted in areas irrigated by erythromycin contaminated waters or in areas where animal farming is extensive.

In conclusion, it would be said that the above methods have low cost and it seems to be effective in the removal or degradation of those compounds. Therefore, further research is necessary to establish a unified method (including biological treatment methods) which will be practically enabled to complete the removal or the degradation of those compounds from waters or soil.

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