Treatment of Cow-Farm Wastewaters Using *Platanus sp*.

Georgopoulos S.[1] And Papadakı M.I.[1*]

[1] Department of Environmental & Natural Resources Management, University of Patras, Agrinio, Seferi 2, 30100, Greece

[*]corresponding author:

e-mail: marpapadaki@upatras.gr

Abstract The modern epoch is characterized by increasing concerns regarding the potentially negative effects for the environment, as a result of human activities. The spectacular progress of industry and technologies as well as the improving of life standards contributed to the introduction of an increasing number of pollutants in the aquatic environment. The ability of plants to remove contaminants from the environment has been researched over the last decades and has found a number of applications. This work presents preliminary findings on the removal-degradation of cows'-farm wastewaters. This work focused on the use of plants and more specifically in plant stems. This method is an attractive treatment option because it is simple to construct and operate, it has low cost and it uses only solar energy. Furthermore, it seems to be effective in the removal or degradation of those wastewaters. However, further research is necessary to establish a synergistic method (including biological treatment methods) which will practically enable complete removal or degradation that compounds from waters or soil.

Keywords: bioremediation, *Platanus* sp., degradation, toxicity.

1. Introduction

Rejection of cow-farm wastewaters without control creates serious environmental problems. Frequent complaints rise, because these wastewaters create environmental pollution at least in surface waters. In an effort to solve the environmental problems induced by these wastewaters, the scientific community is conducting research to establish treatment methods; one of these is phytoremediation (Dietz and Schnoor, 2001).

The ability of plants to remove contaminants from the environment has been researched over the last decades and taken advantage of in applications. Phytoremediation is a newly evolving field of science and technology which uses plants to clean-up polluted soil, water, or air. In other words, it is an ecologically friendly, solar-energy driven clean-up technology, based on the concept of using nature to cleanse nature (Salt *et al*., 1998).

The process of phytoremediation has the potential to treat different types of wastewaters including acid mine drainage, industrial, municipal, agricultural wastewaters as well as tannery wastewaters (Di Luka *et al*., 2011, Calheiros *et al*., 2012, Kongroy *et al*., 2012).

The mechanisms and efficiency of phytoremediation depend on the type of contaminant, bioavailability and soil properties (Cunningham and Ow, 1996). There are several ways by which plants clean up or remediate contaminated sites. The uptake of contaminants in plants occurs primarily through the root system. The root system provides an enormous surface area that absorbs and accumulates water and nutrients essential for growth along with other non-essential contaminants (Raskin and Ensley, 1999). More specifically, these processes include (Susarla *et al*., 2002)

- modifying the physical and chemical properties of contaminated soils,
- releasing root exudates, thereby increasing organic carbon,
- improving aeration by releasing oxygen directly to the root zone, as well as increasing the porosity of the upper soil zones,
- intercepting and retarding the movement of chemicals,
- effecting co-metabolic microbial and plant enzymatic transformations of recalcitrant chemicals and
- decreasing vertical and lateral migration of pollutants to ground water by extracting available water and reversing the hydraulic gradient.

The removal of pollutants from wastewater follows complex mechanisms of reactions which lead to the formation of many intermediate and final products, which are often more toxic to organisms and humans compared to the initial concentrations (Oturan *et al*, 2008), (Rizzo, 2011). Therefore, in purpose to avoid the above disadvantage, it is necessity to find the change of the toxicity of wastewater treatment as a function of treatment time, in order to estimate when the complete removal of the toxicity occurs (Rizzo, 2011).

There are many bioassays to assess the toxicity of wastewater; one of these can be achieved by onions *Allium cepa* L. (Fiskesjo, 1993). This method is an easy and sensitive tool for measuring the total toxicity caused by chemical treatments as expressed by growth inhibition of the roots of onion bulbs. The degree of toxicity of the test chemicals is estimated by measuring the length of the root bundles.

2. Materials and methods

The target of this work is the degradation-removal of cow farm wastewaters with the method of phytoremediation. For this reason, sixteen stems (with length 45 cm) of platanus-*Platanus* sp. were collected from the region of

Kastoria in North-West Greece and more precisely from the south side of Lake Orestidos. The genuses were identified based on keys (Strid and Tan 1997, 2002; http://ww2.bgbm.org/EuroPlusMed/query.asp\).

Four stems, with leaves on the top of each stem, were placed in 2L glass bottles. Two of the glass bottles were filled with 1.5 L of cows'-farm wastewater whereas the other two glass bottles containing 1.5 L of pure tap water (Reference). The stems were collected from the same tree and the distance which they had from the ground was about 1.5-2m. The glass bottles were covered with aluminum foil to prevent photolysis of the wastewater. Furthermore, 1.5 L of cows'-farm wastewater was employed in 2L glass bottle (Reference*) which was also covered with aluminum foil for the same reason. The above action was taken in order to find out if these wastewaters degrade by themselves.The top of each bottle was covered with transparent membrane so as to reduce water losses due to evaporation. The plants were left under natural sunlight but not in direct contact with the sun. The duration of the experiment was four days (05/08/2014-09/08/2014).

On 07/08/2014 stems were replaced with fresh ones, correspondingly. They had 45 cm length, they were collected all from the same tree, but the tress was different from the one employed on the 5th of August; the distance of the stems from the ground was about 2-2.5m. The two trees had a distance of 80-90 m from each other and they were next to the lake.

Before the experiment started, the cows'-farm wastewater has filtered with plastic screen (length: 0.1cm, width: 0.1cm).

In the beginning and at the end of the experiment samples were extracted from each bottle. Each sample was filtered with a HVLP 0.45 μm, 25 mm filter in purpose to remove any particulate matter. The Chemical Oxygen Demand - COD- in each solution was measured. Small volumes of the samples were pipetted into vials containing the premeasured reagent (mercury (II) sulphate and sulphuric acid-84%-). The vials were incubated until digestion was completed in COD reactor (Hach, Model 45600) and then cooled. The COD measurement was made with the DR/890 colorimeter (Hach).

On 09/08/2014 samples were extracted from each bottle to assess the toxicity of the respective aqueous solutions using the onion *Allium cepa* L method to identify if the plants remove or degrade these wastewaters. A series of onions was used for each aqueous solution. In each series 12 glass tubes (12 mL nominal volume) were filled with 11 mL of the respective solution and an onion was placed in the top of each tube, Thiva's commercial onions with zero initial length of roots were used. In addition, two more series were used, one for the initial concentration and one for the control-these onions were placed in enriched with nutrients tap water as shown in Table 1-.

More precisely, Table 1 shows the nutrients used as well as the respective quantities per liter of pure tap water for the control.

In every series, the solution that was absorbed by each onion was replaced every 8 hours, whereas every 24 hours each solution was replaced with respective fresh one. Following the protocol, (Fiskesjo, 1993), after 48 hours, two onions with the most poorly growing roots were removed from each series. After 72 hours, the length of the 10 root bundles was measured in each series by a ruler. Exceptionally short or long roots were ignored.

After 72 hours of the experiment, half of the onions in each series treatment had their solution replaced with the respective fresh solution, whereas for the other half control solution was used. The purpose of the above was to note if the onions can recover the shock to which they were submitted from these wastewaters or in other words to note if the effect was reversed. If root growth is better in the water enriched with nutrients than in the test samples the effect is reversible.

Table 1. The first column represents the nutrients that was used in the control while the second column represents the respective quantities per liter of pure tap water

Nutrients	**Quantities per liter**
$Ca(NO_3)_2 \cdot 4H_2O$	0,0236 g
KNO_3	0,0202 g
$MgSO_4 \cdot 7H_2O$	0,0246 g
KH_2PO_4	0,0135 g
EDTA	0,0220 g
Trace minerals:	
$MnSO_4 \cdot H_2O$	0,550 10^{-6} g
$CuCl_2 \cdot 2H_2O$	0,065 10^{-6} g
$NaMoO_4 \cdot 2H_2O$	0,001 10^{-6} g
$ZnSO_4 \cdot 7H_2O$	0,001 10^{-6} g
H_3BO_3	0,229 10^{-6} g

1. Results and discussion

When the experiment had been completed, the volume of aqueous solutions was measured. These measurements are presented in Table 2. The first column shows the volume reduction and the second the final volume. Initial volume in all cases was 1500 mL. Reference* is the solution without any stem. As can be seen here, there is a small evaporation of the samples. Moreover, the stems in pure tap water (Reference I and II) absorbed four times more water than that of the pure cows'-farm wastewater. The removal-degradation of cow-farm wastewater was determined qualitatively with the onion *Allium cepa* L method. As mentioned earlier, after 72 hours of treatment, the length of the 10 root bundles in each series was measured. The following day, it was examined if the effect of the treated-untreated wastewaters was reversed.The measurements are shown in Table 3. The first column of Table 3 displays the percentage of growth of roots in each sample based on the roots of the control, whereas the second column displays the reversibility of each sample.At the end of the experiment, COD analysis (in mg L^{-1}) of the reported aqueous solutions, of the initial concentration as well as that of the pure tap water was measured. Table 4 presents these measurements. Four measurements had been conducted and the value displayed is the average of them.

From Tables 2-4, can be seen that the results have good repetitability. In addition, Table 2 shows that the stems of Platanus absorbed a large amount of cows'-farm wastewater. The amount which the other pair of stems, submerged in pure tap water absorbed was larger.

Phytotoxicity presented in Table 3, shows that a reduction in the growth of roots of the untreated waste was observed, i.e. the root growth was only 65,63% compared to the root growth of the control. This means that the aforementioned wastewaters are phytotoxic. As can be seen in the rest of the measurements the solution phytotoxicity was not altered with treatment. However, as the volume of the solution was reduced as the result of absorption (Table 2), the overall phytotoxic effects can be considered of lesser importance. Moreover, the phytotoxicity of this wastewater can possibly justify the smaller volume absorption by the stems of *Platanus sp.* as compared with the absorption of pure tap water with nutrients (Table 2). Finally, it is worth mentioning that no sample showed reversibility as the last column of Table 3. So, the onions could not recover the shock which were subjected to from the cows'-farm wastewaters.As can be seen in Table 3 the lengths of the roots of "Reference I" are larger than those of the "Control". This does not mean that the tap water was purified by Platanus, but it only highlights that this method is qualitative and cannot be used for a strict ranking and comparison. Table 4, shows the COD history of the samples. In the last column the COD has been normalized as [COD (final volume)/(initial volume)], to account for the overall reduction of COD of the wastewater volume used. As can be seen there is a reduction of COD in the wastewater which was left untreated. The tap water with stems in it, displayed an increase of COD most likely because of matter transport from the stems to the water. The samples with the stems showed reduced COD removal as compared to the reduction of COD of the untreated wastewaters in both the direct and normalized value. This is possibly owed to the transport of matter from the stems to the solution, to differences in the solution initial composition as these real wastewaters can vary in composition, to the regular addition of fresh wastewater in the bottles with the stems, or to a combination of those.

Table 2. The volume change and the final volume of aqueous solutions. Initial volume 1500 mL

Samples	Volume change of aqueous solution (mL)	Final volume of aqueous solution (mL)
Reference*	21	1479
Reference I	845	655
Reference II	1110	390
Platanus sp. I	224	1276
Platanus sp. II	215	1285

Table 3. Toxicity and reversibility using the method *Allium cepa* L**.** The first column displays the percentage of growth of roots in each sample based on the roots of the control. The second column displays the reversibility of each sample

Samples	% of Control	Reversibility
Control	100	-
Initial	65,63	No
Reference*	93,75	No
Reference I	131,25	No
Reference II	87,50	No
Platanus sp. I	74,22	No
Platanus sp. II	65,63	No

Table 4.COD analysis (mg L^{-1}) of the initial concentration, of the pure tap water as well as of the mentioning aqueous solutions at the end of the experiment

Samples	COD mg L^{-1}	COD_{norm} mg L^{-1}
Initial	967	
Pure tap water	1	
Reference*	300	296
Reference I	15	
Reference II	16	
Platanus sp. I	700	595
Platanus sp. II	400	343

Recently, the removal-degradation of cows'-farm wastewaters was also studied in an other similar work, but this time with microalgae (Ding *et al*., 2015). The duration of that experiment was longer than ours (six days) and the wastewaters were submitted to dilutions of 5, 10 and 20 times. In this work, it was measured the removal of ammonia (NH_3), phosphorus (P) and carbon (C). Specifically, the greatest dilution showed the removal rates: NH_3-99,26%, P-89,92%, C-84,18%. A 10 time's dilution removal was: NH_3-93%, P-91% and C-88%. The 5 times dilution removal was: NH_3-83%, P-92%, C- 90%. A significant removal of all the above chemicals was achieved. The removal of the aqueous solution of the cows'-farm wastewaters was not examined neither did other factors such as nitrogen, potassium.

2. Conclusion

Rejection of cow-farm wastewaters without control creates serious environmentally problems. In this work we tested the degradation-removal of cow farm wastewaters with the method of phytoremediation employing *Platanus sp*. It was found that the stems of this plant substantially absorb wastewaters and substantially reduce its volume. The phytotoxicity removal is not obvious while the COD removal of the wastewaters was significant, but smaller than the respective amount of the sample which was left to degrade naturally. Further work is required for the analysis of specific compounds removed via this species.

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