INTERNATIONAL JOURNAL OF ADVANCED BIOLOGICAL RESEARCH

www.scienceandnature.org

REMOVAL OF PESTICIDE CARBOFURAN USING WETLAND PLANTS

[a*]Shalini A. Tandon, [a]Radhika Deore and [b]Ankita Parab
[a] CSIR-National Environmental Engineering Research Institute, Mumbai Zonal Centre, 89-B,
Dr. A. B. Road, Worli, Mumbai 400018, Maharashtra, India
[b]D.Y. Patil College School of Biotechnology and Bioinformatics Sector 15, Belapur, Navi Mumbai, Maharashtra 400614
*Corresponding Author Email: tandon.shalini@gmail.com

ABSTRACT
Pesticide contamination of the environment has several adverse effects on the ecosystem. Carbofuran is one of the pesticides which is banned or severely restricted in Canada and the European Union, but it is still used in India. In the present study, the wetland plants were used for degradation of Carbofuran. *Chrysopogon zizanioides, Acorus gramineus, and Scirpus cyperinus* plants were used to check their efficacy to remove Carbofuran from water. *Chrysopogon zizanioides, Acorus gramineus,* and *Scirpus cyperinus* plants were treated with 1.5ppm and 2ppm of carbofuran for 15 days. It was found that *Acorus gramineus* was the most efficient plant for the treatment of water contaminated with the pesticide carbofuran up to the concentration of 2 ppm.

KEYWORDS: Carbofuran, Phytoremediation, *Chrysopogon zizanioides, Acorus gramineus, Scirpus cyperinus.*

INTRODUCTION

Pesticide is defined as a substance used for destroying, preventing, repelling or mitigating any pest (USEPA, 2006). Pesticides consist of insecticides, herbicides, fungicides, virucides, and others. The use of pesticides in agriculture is increasing to improve crop production. Per hectare use of pesticide in India is lowest, *i.e.* 0.6 Kg/ha among all the developed and most of the developing countries. As a consequence, the use of pesticide has caused contamination of soil and water components of the environment (Mattina *et al.*, 2006). Moreover the exposure of humans to excess amount of pesticides can be harmful for the health. Insecticides impede the normal functioning of the nervous system. Organochlorine and organophosphates stimulate the nervous system (Vaccari *et al.*, 2006). There is destruction of nerve fibers due to chronic exposure to organophosphates. It also leads to muscle tissue damage (myopathy) (Milatovic *et al.*, 2006). Organochlorines accumulate in the adipose tissue. Physico-chemical methods used to treat pesticides are not efficient and effective. Pesticide residue remains in the soil-water environment and enter the food chain (CFTRI, 2005). Hence, bioremediation techniques are required to degrade pesticides. Carbofuran is an insecticide which is broad spectrum but with a high oral toxicity. In rats the oral LD50 for carbofuran is reported to be 11 mg/kg of body weight. It is used in crops like corn, rice, cotton etc. (Rai *et al.*, 2009) many methods have been employed for degradation of Carbofuran. Phytoremediation is an approach where plants are used remediate polluted soil and water. The plants remove pollutants through selective uptake capabilities of their root systems and their ability to bioaccumulate and degrade the pollutants. In a study, the potential of water hyacinth (*Eichhornia crassipes*) to remove ethion pesticide, was determined. The rate of disappearance of ethion in culture solutions were found to be 0.01059, 0.00930, 0.00294, and 0.00201per hour for the non-sterile planted, sterile planted, non-sterile unplanted, and sterile unplanted treatment, respectively. Plant uptake and phytodegradation contributed towards 69% removal of ethion while microbial degradation contributed towards 12% removal of ethion (Xia *et al.*, 2006). Teerakun *et al.*, (2004) studied the removal of carbofuran residues in rice field soils. The soil samples were analyzed and the results showed that carbofuran degraded rapidly in planted soil. There are no studies involving wetland plants for the treatment of Carbofuran. Thus, this study aims to study the efficacy of wetland plants for removal of Carbofuran.

MATERIALS &METHODS

Spectrophotometric determination of Carbofuran

A stock solution of carbofuran (1 mg/ml) (Sigma-aldrich) was prepared in glacial acetic acid (1:10) and working standard was prepared from it. Diazotized p aminoacetophenone (DPAAP) used was prepared by dissolving 1% PAAP in (1:5) hydrochloric acid. The next step involved addition of 1.5ml of 0.2% freshly prepared sodium nitrite solution. For diazotization, this was kept in an ice bath for a period of 10 min. With the addition of 1mL of 3% sulphamic acid to it excess nitrite was removed (Tamrakar *et al.*, 2007).

Preparation of calibration curve

For the preparation of the calibration curve 2 ml of DPAAP was added to an aliquot containing 4-32 µg of carbofuran (Sigma Aldrich, 98%) in a 25ml volumetric flask. This was kept for a period of 5 min. Followed by the addition of 3 ml of NaOH. This resulted in the formation of an orange yellow dye. The dye was measured at a wavelength of 460 nm and distilled water was used as blank.

Determination of Carbofuran in water

Water samples were extracted with 2 × 10 ml portion of chloroform. The extract was evaporated to dryness. The residue was dissolved in 10 ml of (1:10) acetic acid. DPAAP (2ml) was added to aliquots in 25 ml volumetric flask. Then NaOH (3 ml) was added to it and analyzed at a wavelength of 460 nm.

Acclimatization and Exposure of plants to Carbofuran

Three wetland plants *viz. Chrysopogon zizanioides, Acorus gramineus, Scirpus cyperinus* were selected for the study. Selected plant species were acclimatized for 40 days in modified Hoagland's solution (Tobin *et al.*, 1970). Plants then treated with 1.5ppm, 2ppm of carbofuran for 15 days (Castellanos Rozo *et al.*, 2013; Otieno *et al.*, 2010). Commercial grade pesticide was used for the treatment by taking equivalent amount of Carbofuran 3% GR insecticide. Amount of pesticide removed was estimated by spectroscopic method on alternate days. Plants were weighed before and after the treatment.

RESULTS & DISCUSSION

Acclimatization of plants:

Plants were kept in Hoagland's solution for 25days.

FIGURE 1: Acclimatization of plants Acorus and Scirpus plants

Effect of carbofuran on the physical appearance of plants

Plants were weighed before and after the treatment. No adverse effect of carbofuran was observed on the weight of any plant species, while there was an increase in the weight of *Acorus species* when they were treated with 1.5ppm and 2ppm carbofuran. No adverse impact on the physical appearance of plants was seen after the treatment with carbofuran. The Table 1 summarizes weights of plants before and after the treatment.

TABLE 1: Changes in weight after treatment with Carbofuran

Acorus gramineus			
Treatment of *Acorus gramineus*	Initial weight in grams	Final weight in grams	Increase in weight (g)
Control-no carbofuran	14.49	17.6	2.75
1.5 ppm of carbofuran	10.53	17.15	5.33
2 ppm of carbofuran	19.23	28.85	5.67
Scirpus cyperinus			
Treatment of *Scirpus cyperinus*	Initial weight in grams	Final weight in grams	Increase in weight (g)
Control-no carbofuran	23.67	36.87	12.39
1.5 ppm of carbofuran	24.01	42.94	13.26
2 ppm of carbofuran	32.11	49.36	11.43
Chrysopogon zizanioides			
Treatment of *Chrysopogon zizanioides*	Initial weight in grams	Final weight in grams	Increase in weight (g)
Control-no carbofuran	17.51	29.02	10.86
1.5 ppm of carbofuran	12.83	23.55	9.38
2 ppm of carbofuran	18.28	29.27	11.13

Removal of carbofuran per gram of plants

Plants were treated with 1.5ppm and 2ppm of carbofuran commercial grade pesticide for 15 days. Removal of carbofuran by per gram of the plant was calculated. The removal of carbofuran by per gram of *Acorus gramineus* was found to be 0.05 ± 0.02ppm per day when treated with 1.5ppm as well as 2ppm of carbofuran. Highest amount of carbofuran was removed on the fourth day for 1.5ppm as well as 2ppm *i.e.* 0.06 ±0.02ppm.The removal of carbofuran by per gram of *Scirpus cyperinus* was found to be 0.03 ±0.01ppm when treated with 1.5ppm of carbofuran and 0.04 ±0.02ppm when treated with 2ppm of carbofuran. Thus, mean removal of carbofuran by per gram of *Scirpus cyperinus* is 0.04 ±0.0ppm. By the treatment with 1.5ppm of the pesticide, maximum removal was observed on fourth day *i.e.* 0.04 ± 0.01ppm.By the treatment with 2ppm of the pesticide, maximum removal was observed on fourth day *i.e.* 0.05 ± 0.01ppm.

On an average, removal of carbofuran by per gram *Chrysopogon zizanioides* was found to be 0.04 ± 0.01 ppm when treated with 1.5ppm of carbofuran and 0.04 ± 0.01ppm when treated with 2ppm of carbofuran. Thus, mean removal of carbofuran by *Chrysopogon zizanioides* is 0.04 ±0.01ppm. There is a variation in the removal of carbofuran on the treatment of 2ppm. By the treatment with 1.5ppm of the pesticide, maximum removal was observed on fourth day *i.e.* 0.03 ± 0.01ppm. While, by the treatment with 2ppm, maximum removal was observed on fourth day i.e. 0.06 ± 0.01ppm.

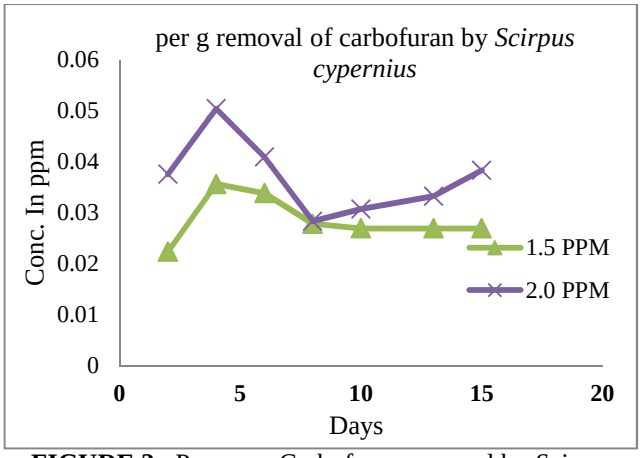

FIGURE 2 : Per gram Carbofuran removal by *Scirpus cypernius*

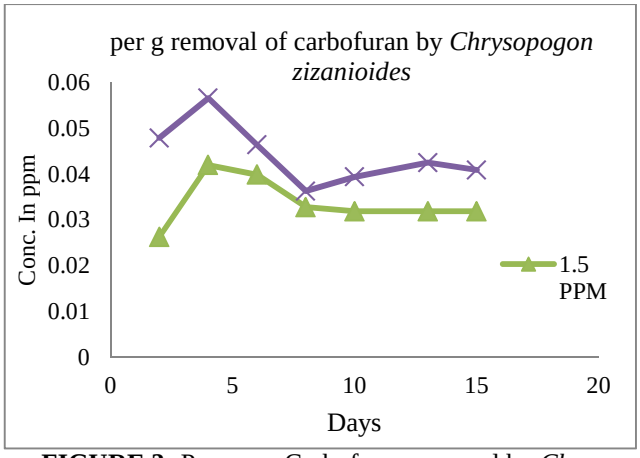

FIGURE 3: Per gram Carbofuran removal by *Chrysopog zizanioides*

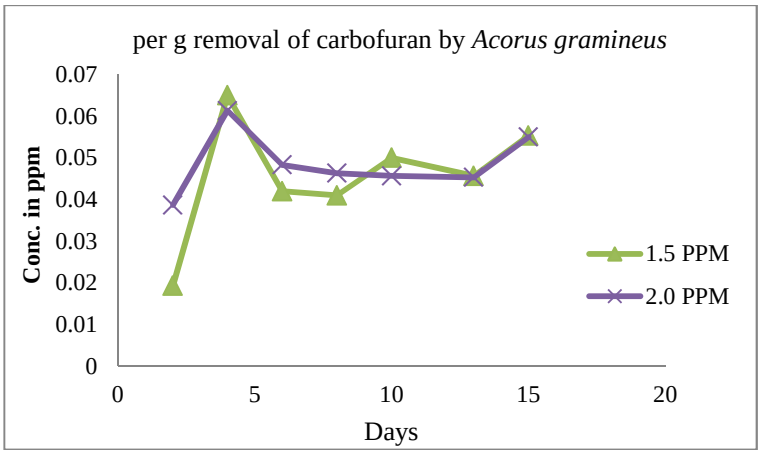

FIGURE 4 : Per gram Carbofuran removal by *Acorus gramineus*

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.001045	2	0.000523	6.222224	0.008828	3.554557
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.000482	2	0.000241	4.69814	0.022815	3.554557

The removal efficiency of three selected species at a particular pesticide concentration was compared to determine the most efficient plant species for pesticide removal. *Acorus gramineus* showed maximum removal, while the removal of carbofuran by *Scirpus cyperinus* was least when treated with 1.5ppm of carbofuran. ANOVA performed showed the significance level of value 0.009 which is below 0.05 and, therefore, there is a statistically significant difference in the removal of carbofuran by selected plants. *Acorus gramineus* is showing maximum removal, while rate of removal of carbofuran by *Scirpus cyperinus* is least when treated with 2ppm of pesticide. ANOVA performed showed the significance level of value 0.023 which is below 0.05 and, therefore, there is a statistically significant difference in the removal of carbofuran by selected plants.

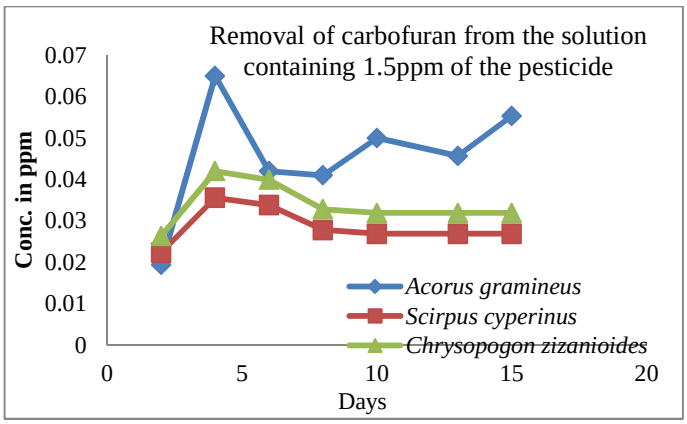

FIGURE 4: Per gram Carbofuran removal at 1.5ppm

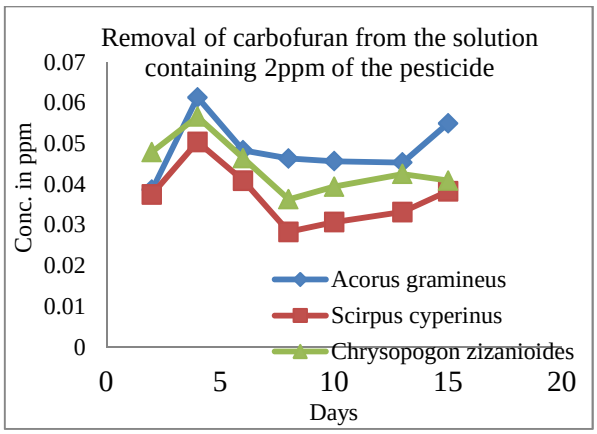

FIGURE 5: Per gram Carbofuran removal at 2ppm

CONCLUSION

Carbofuran up to 2ppm has no adverse impact on the physical appearance of plants *Acorus gramineus, Scirpus cyperinus, Chrysopogon zizanioides* after the treatment. Plants were able to remove maximum amount of carbofuran in first four days of the treatment. *Acorus gramineus* is the most efficient plant for the treatment of water contaminated with the pesticide carbofuran up to the concentration of 2ppm.

Acknowledgement: KRC No.: CSIR-NEERI/KRC/ 2016/ OCT/MZL/1

REFERENCES

Castellanos Rozo, J., Sánchez Nieves, J., Uribe Vélez, D., Moreno Chacón, L., &Melgarejo Muñoz, L.M. (2013) Characterization of carbofuran degrading bacteria obtained from potato cultivated soils with different pesticide application records. *Revista Facultad Nacional de Agronomía, Medellín*, *66*(1), 6899-6908.

Central Food Technological Research Institute (2003) Report of analysis of pesticide residues in soft drink samples, Mysore, India.

Mattina M., Berger W., Musante C., and White I. (2006) Phytoremediation of contaminated soil and groundwater at hazardous waste sites. *Bioremediation journal. Vol.* 1(3), 62-69.

Milatovic, D. Gupta, R.C. and Aschner, M. (2006) Anticholinesterase Toxicity and Oxidative Stress. The Scientific World Journal 6, 295–310

Otieno, P.O., Lalah, J.O., Virani, M., Jondiko, I.O., & Schramm, K.W. (2010) Soil and water contamination with carbofuran residues in agricultural farmlands in Kenya following the application of the technical formulation Furadan. *Journal of Environmental Science and Health Part B*, *45*(2), 137-144.

Rai, D, Rai, P., Gupta, A., Watal, G., Sharma, B. (2009) cartap and carbofuran induced alterations in serum lipid profile of Wistar rats. Indian Journal of Clinical Biochemistry, 24 (2) 198-201.

Tamrakar, U., Pillai, A.K., & Gupta, V.K. (2007) A simple colorimetric method for the determination of carbofuran and its application in environmental and biological samples. *Journal of the Brazilian Chemical Society*, *18*(2), 337-341.

Teerakun, M., Reungsang, A. & Virojanakud, W. (2004) Phytoremediation of carbofuran residues in soil. *Song klanakarin J. Sci. Technol*, 26(Suppl 1), 171-176.

Tobin, J.S. (1970) Canbofuran: A New Canbamate Insecticide. *Journal of Occupational and Environmental Medicine*, *12*(1), 16-19.

USEPA 2006

Vaccari, Strom and Alleman (2006) Environmental Biology for Engineers and Scientists.

Xia, H. & Ma, X. (2006) Phytoremediation of ethion by water hyacinth (*Eichhornia crassipes*) from water. *Bioresource Technology*, *97*(8), 1050-1054.