

Triclosan Persistence in Environment and Its Potential Toxic Effects on Algae

Saif Al Ghais, Vibha Bhardwaj & Pramod Kumbhar

Environment Sustainability Department, Environment Protection & Development Authority (EPDA), Ras Al Khaimah, UAE.



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ABSTRACT

Triclosan (TCS) is widely used as an antibacterial agent in various industrial products, such as textile goods, soap, shampoo, liquid toothpaste and cosmetics, and often detected in wastewater effluent. Triclosan is highly toxic to aquatic animals, and is particularly highly toxic to the algae, which was used as a test organism in this study. Algae represent the first nutritional base on the aquatic food chain due to their ability to synthesize organic molecules using sunlight and carbon dioxide. Thus, the cultivation of algae has been an integral part for the production of commercially important species on aquaculture. This has been the reason to make great efforts in order to understand effect of triclosan to natural periphyton communities (algae). However, there is a paucity of data on the toxicity of triclosan and its effects on aquatic organisms. In this study, the toxicity of triclosan to a microalga was examined. The present investigation showed that “chlorophyll a” pigment in control algae was 5.92 mg/L and it decreased, when algae was treated with different concentration of triclosan (10-50 ppm). The study revealed that, as the concentration of triclosan increased then algae growth declined. It was observed that conductivity also increased because of decreased “chlorophyll a” and decreased phytoplankton levels. This result indicates that triclosan exerts a marked influence on algae, which are important organisms being the first-step producers in the ecosystem; therefore, the possible destruction of the balance of the ecosystem is expected if triclosan is discharged into the environment at high levels. The bioaccumulation of TCS in human impregnation from foodstuff exposure (in particular fish) and likely risk for human population also.

Keywords: Triclosan, Algae, Growth curve, Chlorophyll, Conductivity, Aquatic environment.

1. Introduction

Triclosan (TCS) is considered as a ubiquitous pollutant, detected in all types of environmental compartments including aquatic environments (lakes and rivers, coastal and estuarine waters, wastewater treatment plants (WWTP)), domestic and drinking water, soils and bio solids (sediments and sludge), indoor dust, living organisms (fish) and humans (Chu and Metcalfe 2007; Chalew and Halden 2009; Reiss et al. 2009). It has been used in consumer products since 1968 as an antiseptic, disinfectant, and preservative in clinical settings; various consumer products including cosmetics, household cleaning products and toys; and it has also been incorporated on the surface of medical devices, plastic materials, textiles, and kitchen utensils. TCS released to the environment is acutely and chronically toxic to aquatic organisms (Bhardwaj et al, 2016). However, TCS was removed in 2010 from the EU list of additives for use in plastic use food-contact materials (Commission decision 2010/169/EU). On September 9, 2016, the United States Food and Drug Administration (FDA) banned the incorporation of triclosan and 18 other antimicrobial chemicals from household soap products and the next year prevented companies from using triclosan in over-the-counter health care antiseptic products without premarket review. On the other hand, about 85% of the total volume of TCS is used in personal care products, compared to 5% for textiles and 10% for plastics and food contact materials (SCCS 2010). The regulation of cosmetic products differs according to the country where the product is marketed. During the last decade, and even quite recently (Brausch and Rand 2011), TCS has been the target of several studies focused on the evaluation of its fate and distribution in the environment (all types of water, living organisms, soil, sludge, sediment, etc.), indoor environment (dust), and human samples. With its widespread use of TCS and mostly “down-the-drain” and to surface waters where it may finally reach humans by drinking contaminated water or via food chain by consumptions of animals and vegetation

exposed to TCS. Due to improvements in analytical methods and environmental distribution studies, TCS has been found in many environmental samples. In this research work, we focus on the toxicity of TCS to aquatic organisms. Microalgae represent the first nutritional base on the aquatic food chain due to their ability to synthesize organic molecules using sunlight and carbon dioxide (Fuentes-Grünewald et al., 2012). Algae form the base of aquatic food webs and play important roles in energy and nutrient transfer to upper trophic level species. In addition, algae have proved to accumulate many pollutants from the water which can be transferred to species at higher trophic levels. Algae have a fast reproduction rate and high sensitivities to environmental disturbance and pollution. Microalgae could be used as live food for various stages of growth in marine filter feeders (Ferreira et al., 2008), as food for larvae of some gastropods (during their juvenile stages), and also as food for some crustaceans and some fish species in its earliest growth stages (Brown and Robert, 2002). Microalgae are also used as indirect food when used in the zooplankton production (i.e. artemia and rotifers), essential food for several carnivorous larvae (Welladsen et al., 2014).

The increasing concern about the emerging contaminants, especially the endocrine disrupting chemicals, which include pesticides and the pharmaceuticals and personal care products, has stirred up the rate of monitoring their presence in the environment. Therefore, our study purposed to investigate the effects of triclosan on aquatic organisms and its influence on algae, which are important organisms being the first-step producers in the ecosystem; therefore, the possible destruction of the balance of the ecosystem is expected if triclosan is discharged into the environment at high levels.

2. Materials and Methods

2.1 Micro-algal strains and culture

The micro-algal *Nannochloropsis* sp. used was obtained from the Arabian Gulf Culture Collection of Algae of EPDA. Starter cultures of 100 mL were incubated under a 12 h/12 h L/D (Light/Dark) regime at 50–80 IE/m²/sec at 20 °C for 7– 10 d, without shaking (Westpoint Int. WPX-287.TG, China). Once the culture reached stationary phase, they were harvested by centrifugation at 4000g for 15 min (Sigma 3- 30KS centrifuge, Germany). The harvested cells were then stored in the dark. The purity of algal stock was frequently examined under microscope (Al Ghais et al 2018).

2.2 Preparation of Triclosan standard solutions

A Standard stock solution of triclosan (1000 ppm) was prepared by weighed accurately 100 mg of triclosan standard to 100 mL with methanol and dilute up to mark with methanol. From the stock solution, five different concentrations of triclosan solution were prepared i.e. 10ppm- 50ppm in methanol.

2.3 Treatment of algae with Triclosan

The cell concentration algal culture was adjusted from the algal stock in a 10 mL centrifuge tube. Algal culture (500µl) solution was transferred to each freshly prepared concentration of triclosan solutions (2 mL) to achieve the nominal exposure concentrations, each containing the diluted algal solution for a treatment in the exposure. The experiment was done in triplicate.

2.4 Measurement of effect of different concentration of triclosan on Algae

Absorbance of light by a suspension can be related directly to cell density using a suitable standard curve. Culture samples (2 ml) were centrifuged in Eppendorf tubes at 10,000 rpm for 3 min and the supernatant was used to take the absorbance. The relationships between absorbance and algal concentration (cell density) were assessed in all experimental tubes for 5 days. For each analyzed algae, the absorbance was inspected at 635nm by spectrophotometer (Oak Ridge laboratory guidelines, USA), by using fresh algae culture with methanol but without triclosan as control.

2.5 Quantification of Chlorophyll a

The chlorophyll content was estimated according to the method of Arnon (1949) and Jeffrey et al (1997). Pigments were extracted from algal cells using methanol (A09A/0409/0801/21, SD FCL, Germany). Culture samples (2 ml) were centrifuged in Eppendorf tubes at 10,000 rpm for 3 min and the supernatant was used to quantify chlorophyll a. The absorbance was determined at 664nm by spectrophotometer and the concentration of chlorophyll a, was calculated (Jeffrey et al., 1997). To compare the quantification of chlorophyll concentration, between treated and nontreated, fresh algae culture was used as control. All measurements were carried out in triplicate. The chlorophyll content was expressed in mg/L.

2.6 Growth curve of algae

Sterile algal culture was used to study the growth curve. To study the effect of triclosan on algae growth curve, experiments were performed to compare the growth curve between control (without triclosan) and treated culture (with triclosan). The OD was taken at 665nm by spectrophotometer (Wellburn, 1994). All measurements were carried out in triplicate.

2.7 Quantification of Conductivity

Sterile algal culture was used to study the conductivity effect. To study the quantification of conductivity, experiments were performed, to compare the conductivity of different concentration solution of triclosan and conductivity of different triclosan solutions with algae by using multimeter (Flöder et al.; 2010 and Larson et al 2014). All measurements were carried out in triplicate. The conductivity was expressed in $\mu\text{S}/\text{cm}$.

2.8 Statistical analysis

All experiments were carried out in triplicate. Data are expressed as mean. Pair wise comparisons were performed. Experimental error was determined for triplicate assays and expressed as standard deviation (SD).

3. Results and Discussion

Triclosan (TCS) is detected in all types of environmental compartments including aquatic environments (lakes and rivers, coastal and estuarine waters, wastewater treatment plants (WWTP)), domestic and drinking water, soils and bio solids (sediments and sludge), indoor dust, living organisms (fish) and humans (Chu and Metcalfe 2007; Chalew and Halden 2009; Reiss et al. 2009). Many consumer product chemicals are disposed of, and making it

important to understand the potential for effects of TCS on aquatic organisms. Because a small portion of these chemicals produced potential effects on aquatic organisms.

In the present study, visual changes, conductivity, measurement of effect of different concentration of triclosan on Algae, quantification of “Chlorophyll a” concentration, growth curve was investigated.

3.1 Measurement of effect of different concentration of triclosan on Algae

According to the present research findings, it was observed that the population of algae, significantly decreased as the concentrations of the triclosan increased in comparison to the control (figure 1). The less the algal cells present in solution, the less turbid the solution. Less turbid solution allowed light to pass through the sample and resulted in more transmittance. An interesting thing that caught my eye was that, the color of the algae was also being affected by the triclosan, it was observed that the increase of triclosan, also caused the algae colour change and it appeared, grey. Similar results were reported by David et al 2015. According to Bedoux et al 2012, that there is toxicity of antimicrobial triclosan on environment.

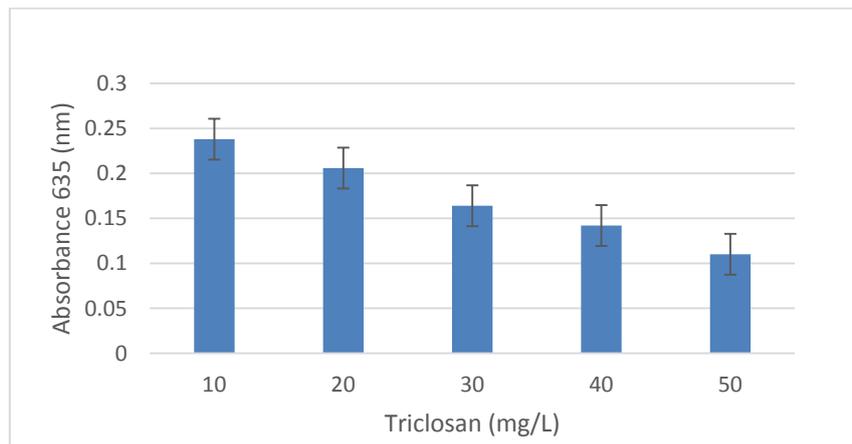


Figure 1: Effect of different concentration of Triclosan on Algae Growth

3.2 Quantification of Chlorophyll a

The chlorophyll content was estimated according to the method of Arnon (1949) and Jeffrey et al (1997) and it was observed that as the concentration of triclosan increased, the amount of “chlorophyll a”, decreased as compared to control. It was also observed that every day the concentration of chlorophyll decreased. In control the concentration was 5.92 mg/L. On day 1, at 10ppm the concentration was 5.61 mg/L but on day 5 it was observed 1.85 mg/L (Table 1). Similar results were reported by David et al 2015.

Table 1: Result of Concentration of “Chlorophyll a”

| Concentration of Chlorophyll a (mg/L) | | | | | | |
|---------------------------------------|--------|--------|--------|--------|--------|---------|
| Days | 10 ppm | 20 ppm | 30 ppm | 40 ppm | 50 ppm | Control |
| Day-1 | 5.61 | 4.30 | 4.00 | 3.71 | 3.30 | 5.92 |
| Day-2 | 2.93 | 2.79 | 2.67 | 2.12 | 2.03 | 5.92 |

| | | | | | | |
|--------------|------|------|------|------|------|------|
| Day-3 | 2.42 | 2.26 | 2.19 | 2.01 | 1.93 | 5.92 |
| Day-4 | 2.27 | 2.03 | 1.86 | 1.77 | 1.38 | 5.92 |
| Day-5 | 1.85 | 1.68 | 1.13 | 0.98 | 0.81 | 5.92 |

On day 5, at 50ppm, the concentration of chlorophyll was observed 0.81 mg/L (figure 2). This same trend of decrease in chlorophyll was observed in all concentrations for 5 days as compared to control.

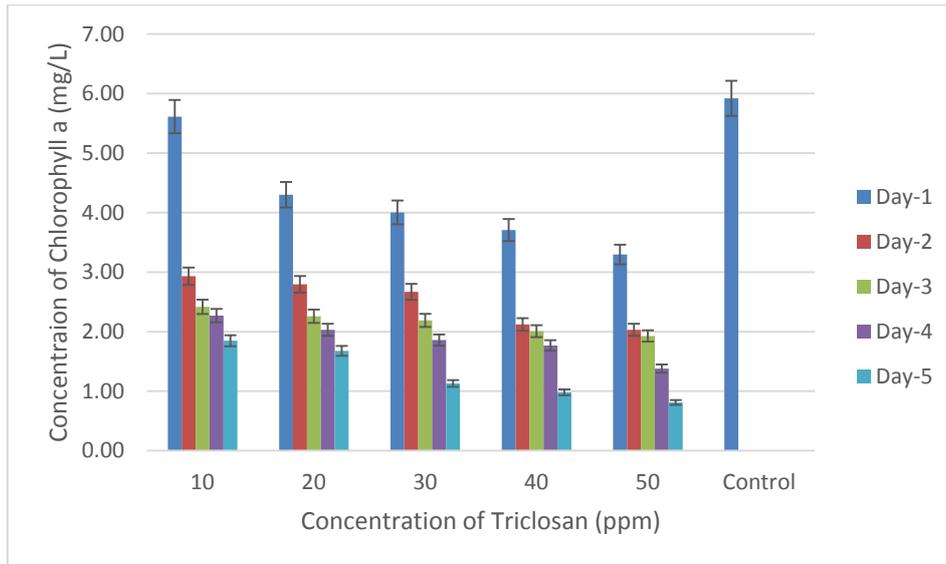


Figure 2: Concentration of “Chlorophyll a” (mg/L) after treatment with triclosan (ppm)

3.3 Growth curve of algae

A growth pattern was studied for algae in comparison with triclosan treated (50ppm). It was observed that the exponential phase came and was not for long duration in triclosan treated algae but in control the growth pattern was normal.

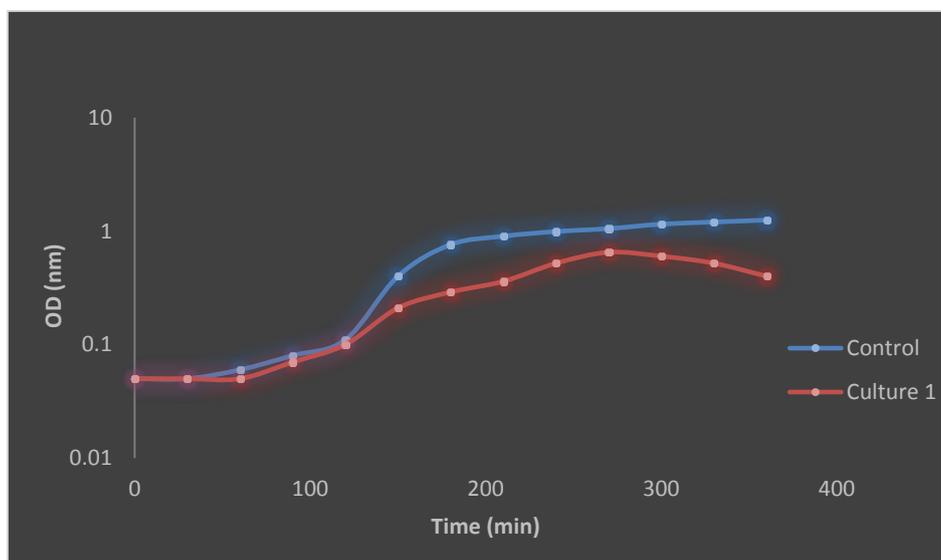


Figure 3: Growth Curve of algae in comparison with control and triclosan treated algae (50 ppm)

It was also observed that there was no stationary phase and, abruptly decline phase started in triclosan treated algae as compared to control (figure 3). Similar results were given by Ran bi et al 2018.

3.4 Quantification of Conductivity

According to our research findings, it was observed that as increased conductivity will correlate with decreased chlorophyll *a* concentration. These research result showed that increases in conductivity may result in decreased phytoplankton levels. Similar results were reported by Flöder et al.; 2010 and Larson et al 2014 (Table 2).

Table 2: Quantification of Conductivity, before and after algae treatment.

| S. No. | Concentration in ppm | Conductivity in $\mu\text{S}/\text{Cm}$ before treatment | Conductivity in $\mu\text{S}/\text{Cm}$ after algae treatment |
|--------|----------------------|--|---|
| 1 | 10 | 7.2 | 155.6 |
| 2 | 20 | 7.0 | 130.2 |
| 3 | 30 | 6.6 | 158.4 |
| 4 | 40 | 6.5 | 132.1 |
| 5 | 50 | 6.3 | 132.8 |

4. Discussion

According to the present research findings, it was observed that the population of algae, significantly decreased as the concentrations of the triclosan increased in comparison to the control, Similar results were reported by David et al 2015. According to Bedoux et al 2012, that there is toxicity of antimicrobial triclosan on environment. The negative impact of TCS on ecosystem is expected to have economic consequences. The United Nations Food and Agriculture Organization (FAO) reports (FAO 2014) that about 25 million tonnes of seaweeds and other algae have been harvested annually for use as food, in cosmetics and for fertilizers, and are processed into thickening agents or animal feed additives. Given the negative effects of TCS on the aquatic flora and fauna such as algae and fish, all the economic advantages such as protein supply from water resources as well as employment provision, risk shortages if TCS circulation is not properly regulated. Results of Bhardwaj et al 2016, also showed that there is harmful effect of triclosan on environment and agriculture.

5. Conclusion

This study concluded that the occurrence of TCS in various environmental media, human body and in wildlife show that the compound is not well regulated. Its uncoordinated use and careless disposal may threaten lives and the ecosystem generally. Cell based studies have shown toxicity potentials of TCS in algal cells.

On basis of our research analysis, Interestingly, even at minute concentrations, triclosan can have a potent effect on the population of algae. According to our research results, the triclosan significantly decreased the algae population as concentrations of triclosan increased. What was especially surprising was that on the last testing at the highest

concentration, there were several instances where there was very less trace of algae population left, so we can conclude the potential effect of this chemical. With these research results we can see that the accumulation of triclosan in our waters which can lead to the corruption of the algae population in the environment which in effect can disrupt the cycle of the ecosystem.

6. Abbreviations

TCS: Triclosan **ppm:** parts per million **WWTP:** wastewater treatment plant **FDA:** food and drug administration **SD:** standard deviation.

Declarations

Ethical approval and consent to participate

Not applicable.

Availability of data and materials

The relevant data and materials are available in the present study.

Competing interests

The authors declare that they have no competing interests. All procedures followed were in accordance with the ethical standards (institutional and national). All institutional and national guidelines for the care and use of laboratory animals were followed.

Funding

Not applicable.

Authors' contributions

SAG supervised the entire project. Supervision of the laboratory work was performed by VB. VB analysed the data and wrote the manuscript. VB did all experiment work. PK assist the experiment work.

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