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Keywords: *artemia cyst; acute-term mortality; ecotoxicology; hatching test; tributyltin chloride.*

GJMR-B Classification: *NLMC Code: QV 55*

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Keywords: artemia cyst; acute-term mortality; ecotoxicology; hatching test; tributyltin chloride.

I. Introduction

Recently there are many research about the acute toxicity of Tributyltin Chloride (TBTCl) on marine organisms. In this research studying the acute toxicity tests on *Artemia* sp. in previous studies focused on a nauplii of *Artemia* sp. This research aims to investigate the toxicities of (TBTCl) of the hatching stage of dry cysts and morphological changes of newly hatching nauplii of *Artemia salina*. In most scientific research widely used *Artemia* sp. as a model marine organism for ecotoxicity test, due to it is large geographical distribution. Despite it is popularity, the use of *Artemia* sp. in toxicity check is subjected to a wide discussion, at the global level, more often than not due to a number of criticisms about low sensitivity and lack of protocol standardization. (George-Ares et al., 2003; Mayorga et al., 2010; Leis et al., 2014; Libralato, 2014 and Rotini et al., 2015). Biological influences of TBTCl on *A. salina* may additionally furnish clues for of the accumulation mechanisms in coastal ecosystems as nicely as of the mode of action of TBT in these organisms. *A. salina* and different Artemia species have been used in the literature for the screening of acute toxicities of booster biocides (Bartolomé and Sánchez-Fortún, 2005; Koutsaftis and Aoyama, 2008 and Rotini et al., 2015). There are many advantages to use Artemia for example, adaptability to high temperature, adaptability to wide ranges of salinity, adaptability to varied nutrient resources, ease of culture, small body size and short life cycle (Nunes et al., 2006 and Koutsaftis and Aoyama, 2008). In addation, *Artemia* is low cost and can use it anywhere at any time.

Tributyltin chloriad is environmental hazards. The half of lives of tributyltin in the marine surroundings had been reported as nearly a number of days to weeks in water and frome one to ten years in sediments (Huang et al., 2004 and Al-Rashdi, 2011). In the previous studies toxicities of booster biocides have been reported on embryos of some marine organisms such as freshwater mussels, zebra mussels, blue mussels, sea urchins, oysters, and sea squirts (Bellas et al., 2007; Fent, 1996 and Wang et al., 2012). High concentrations of BTs have been detected in lower trophic animals such as caprellids. It appears that TBT accumulates specially in caprellids in the marine ecosystem, irrespective of the trophic level in the food

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chain, and that it may additionally establish breakpoint for disturbance in the herbal meals chain structure, therefore inflicting different organisms to accumulate TBT at increased concentrations due to their lower metabolic capacities to degrade TBT (Ohji et al., 2002a). in most of study about TBTCl was recognized as an environmental hazard and can effects on the early embryonic development of *Artemia salina*. They have proven toxic to embryos and larval stages of numerous aquatic organisms even at environmentally concentrations (Bryan and Gibbs, 1991). TBTCl a biocide added to antifouling paints to prevent the accumulation and attachment of algae and barnacles on the bottom of boats. TBTCl has become popular because it is effective, for a long time and causes environmental hazards to non-targeted estuarine organisms. This study aimed to investigate the effect toxicities of tributyltin chloride on hatchability cysts and study morphological changes of newly hatching nauplii of *A. salina*. And observe the morphological abnormalities of *A. salina* newly nauplii of completely hatched in each concentration of TBTCl toxicant and measurement the total length and width of the body (head width, abdominal width and tail width).

II. Materials and Methods

a) Hatching procedure and acute toxicity tests

The tributyltin chloride (TBTCl) used in toxicity tests was kindly provided by Sigma-Aldrich, USA (purity 96%). Stock solutions of TBTCl were prepared by diluting with artificial seawater up to 35‰ salinity. The range of concentration TBTCl was selected as (5, 10, 15, 20, 25, 50, 75, 100, 150, 200 ngl^{-1}). The experiments were performed in 50 ml test tube within tube racks that were submerged in water up to the midpoints of the tubes. Constant aeration, illumination (1000 Lux), and temperature (28 ± 1 °C) were maintained and the replicate number was three in the experiments each replicate 100 cysts of *A. salina* cysts. For each test group, added 40 ml from the test solution of different concentration TBTCl to test tubes, and then a 24 hours hatching period was initiated. After the hatching period, the number of newly hatched nauplii, viable hatched, cysts, and malformation newly hatched nauplii were counted. Hatching percentages (HPs) of cysts were determined by counting the number of completely hatched of nauplii. Hatching failure (found by subtracting the number of completely hatched nauplii from total group size). After that account hatchability (%), Deformity (%) and viable hatchability (%) by using the following formulae (Revathi and Munuswamy, 2010).

Hatchability (%) = 100* (no. of hatched larvae) / (no. of total cyst in test)

Deformity (%) = 100*(no. of deformed larvae)/ (no. of hatched larvae)

Viable hatchability (%) = 100*(no. of viable hatchability larvae in test)/ (no. Total cyst in test).

b) Median effective concentration (EC_{50})

The data on the hatchability inhibitor % of cysts was used in the estimation a 50% effective concentration (EC_{50}) in different concentration of TBTCl. The effective concentration EC_{50} values were determined by using probit analysis in XL TEST-Pro (version 2014.5.03). And each end point was calculated by using the following formulae (Shimasaki et al., 2003).

c) Morphological abnormalities

The morphological abnormalities of *A. salina* newly hatched nauplii of completely hatched in each concentration exposed of TBTCl toxicant were observed under magnification (10x) using a Leica M 205 stereomicroscopy attached to a camera with an aid of software (Easy-Grab; Noldus Information Technology) and the total length and width of the body (head width, abdominal width and tail width) have been measured (Alyuruk and Cavas, 2013).

III. Results and Discussion

Effects of Tributyltin Chloride on Cysts Hatchability in Artemia salina

1. Hatching Percentages (%)

Hatchability of the exposed *A. salina* cysts to different concentrations of TBTCl observed in this study is presented in *Figure 1*. The hatching percentages were shown to be affected by TBTCl concentrations. TBTCl significantly reduced hatching percentages of *A. salina* cysts at the various concentrations by using the following formulae (Revathi and Munuswamy, 2010). Hatchability (%) = 100* (no. of hatched larvae) / (no. of total cyst in test). The hatching percentages in the control were 97%, which is within the reported value of the manufacturer (minimum of 90% hatchability). Among these completely hatched cysts, 76% were active and 21% were viable hatched (completely hatched, but still not active). The remaining 3% cysts were found hatched after hatching period was prolonged for more than 24 to 48hr. From the results observed a significantly decrease hatching percentages from the 5 to 200 ngl^{-1} and was TBTCl had varying effects on the hatching percentages of *A. salina* cysts (Figure 1). In 200 ngl^{-1} showed complete hatching inhibition percentages of *A. salina* cysts. The cysts exposed to TBTCl within 24hr were unable to hatch even the hatching period was prolonged until 48hr. This result confermed TBTCl can kill a embryo and inhibit hatchability of *A. salina* cysts.

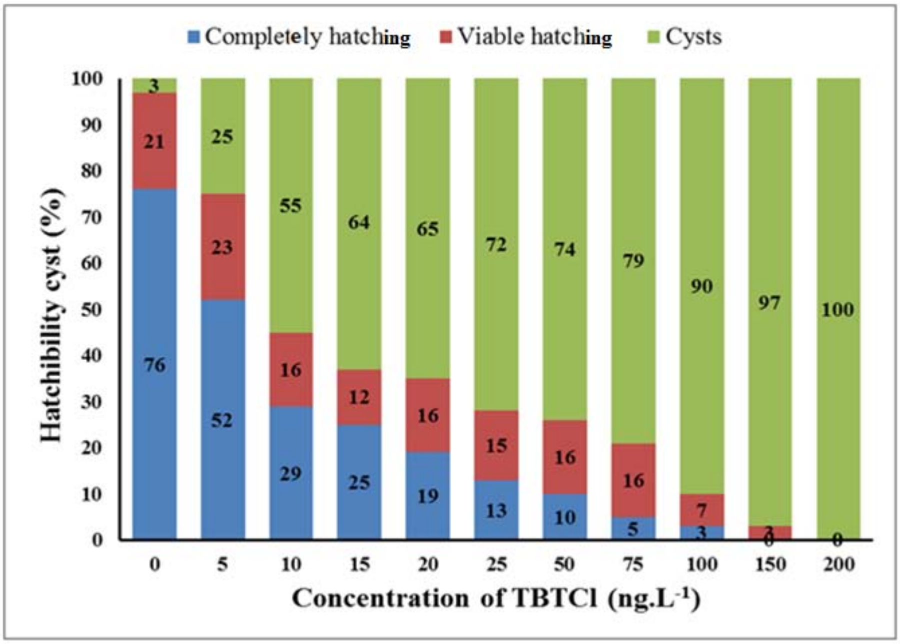

Figure 1: The cysts hatchability percentage of *A. salina* after exposure for 24-h in different TBTCl concentrations [Completely hatching = active nauplii, Viable hatching = nauplii in membrane embryo, Cysts = unable to hatch]

A. salina cysts are a barrier to withstand external environmental condition. Disruption of these activities by certain concentration of TBTCl bioaccumulated into the cysts may cause death to the dormant embryo, and this finding is relative agreement with the results of this research have shown that the toxicity of TBTCl can impact the hatching process of *A. salina* cysts. This result is supported through Brix et al., (2006) studied estimated the median high-quality concentrations (EC_{50}s) for metallic salts, suggesting that the hatching end point for *A. franciscana* is the most touchy examined to date for steel salts in saline environments and same in sensitivity with the most touchy tested to date for Cu. But in present finding *A. salina* cysts are more sensitive to TBTCl at lower concentration 5 ngl^{-1} it was 25% cysts comparative with the control samples 3% cysts, and that mean TBTCl can inhibits hatching process and can kills dormant embryo in the low concentration and also when increasing the TBTCl concentration. Revathi and Munuswamy, (2010) investigated the effects of TBT on the embryonic development, and hatching success of eggs uncovered to TBT in the freshwater prawn brooder *Macrobrachium rosenbergii*, and observed TBT at 3.12 ppm, delayed the embryonic development and significantly reduced the hatchability of eggs as well. two on the different hand, the treated businesses showed impaired embryonic development with reduced body growth. Thus, TBT has appreciably retarded the embryonic improvement in the freshwater prawn *M. rosenbergii*. These studies clearly demonstrated the possible effects of toxicants particularly TBTCl on unhatched eggs or cysts of crustaceans, including *A. salina* was more sensitive to TBTCl at 5 ngl^{-1}, and the possible reason that TBT is more toxic to *A. salina* because the body size is small and it is life cycle is very short *Figure 2* shows effect of different concentrations of TBTCl on the performances hatching of *A. salina* cysts percentages. *Figure 2 (a)* shows the completely hatching (%) that mean the newly hatching nauplii is active and healthy as shown in control. Several nauplii exposed to 10, 25 and 50 ngl^{-1} wereviable hatching (%) that mean the newly hatching nauplii completely hatching, but still not activeand in the embryonic membrane (*Figure 2 (b), (c) and (d)),* while *A. salina* cysts exposed to 75 ngl^{-1} TBTCl concentration was unable to completely break the cyst wall (*Figure 2 (e)*), while the *A. salina* cysts in the 100 ngl^{-1} unable to hatching (*Figure 2 (f)*). This sequence of effects relatively showing the severity of TBTCl as its concentration increase in the aquatic environment.

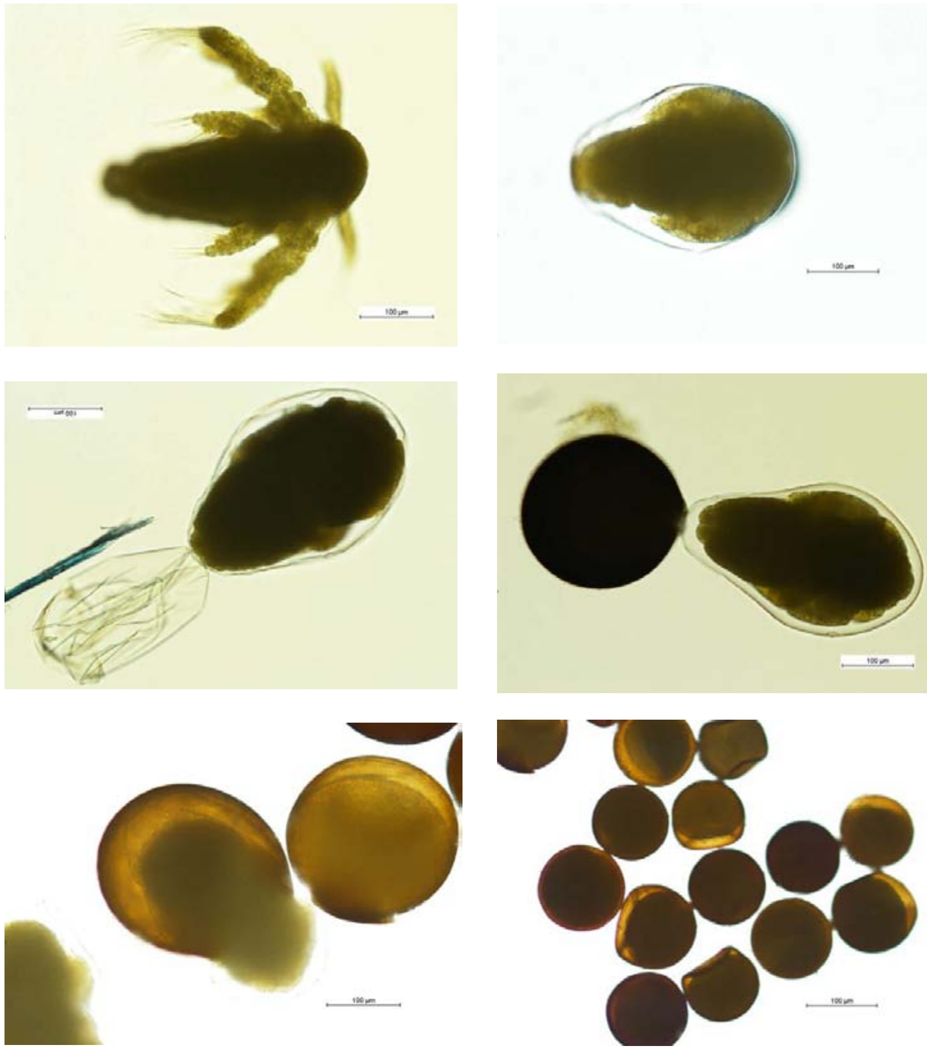

Figure 2: Effects of Increasing TBTCl Concentration on the Performances Hatching Percentages (%) HPs of *A. salina* Cysts. [Remark: (a) control = Completely hatching, b) 10, c) 25, and d) 50 ngl^{-1} = Viable hatching, e) 75 ngl^{-1} = unable to complete break the cysts wall, and f) 100 ngl^{-1} = unable to hatch *A. salina* cyst. [Bar: 100 μm].

2. Median Effective Concentration (EC_{50}).

The median effective concentration EC_{50} of TBTCl as shown in (*Figure 3*), at different concentration of TBTCl the *A. salina* nauplii completely hatching after 24hr was (EC_{50} 46.48 ngl^{-1}), this is mean the TBTCl impacted the process of hatchability *A. salina* cysts and significantly reduced the hatchability cysts when increasing the concentration of TBTCl. Since there is a confined research on the inhibitory effects of the hatchabilty share cysts of *A. salina* it was once two examine EC_{50} of TBTCl with one of a kind toxicants such as metals have been pronounced on the hatchability percentage of cysts for instance (Caldwell et al., 2003) studied *A. salina*, have been observed to inhibit hatching success of *A. salina* cysts in dose. A higher sensitivity was once discovered in the 24 and 72hr publicity EC_{50} for 24hr was once 2.14 and 72hr was 0.023 μg ml^{-1}. This result is an settlement with (Brix et al., 2006) studied estimated the EC_{50} of metallic salts are suggesting that the hatching endpoint for *A. franciscana* is the most sensitive examined to metals in marine environments. Meanwhile, Alyűrűk and Çavaṣ, (2013) mentioned their investigation related to the toxicities of diuron to the hatching stage of *A. salina*, their results showed that diuron should be a attainable hatching enzyme inhibitor and used to be substantially lowered the hatching proportion of *A. salina* cysts and prevented the hatching of cysts. Rotini et al., 2015 said in their learn about *Artemia* sp hatching assay is a touchy choice device to acute toxicity take a look at and the hatching test resulted extra touchy than acute mortality tests. The outcomes show the reliability and excessive sensitivity of this hatching assay on a short time and guide it is a useful application of first tier risk assessment techniques in the marine environment.

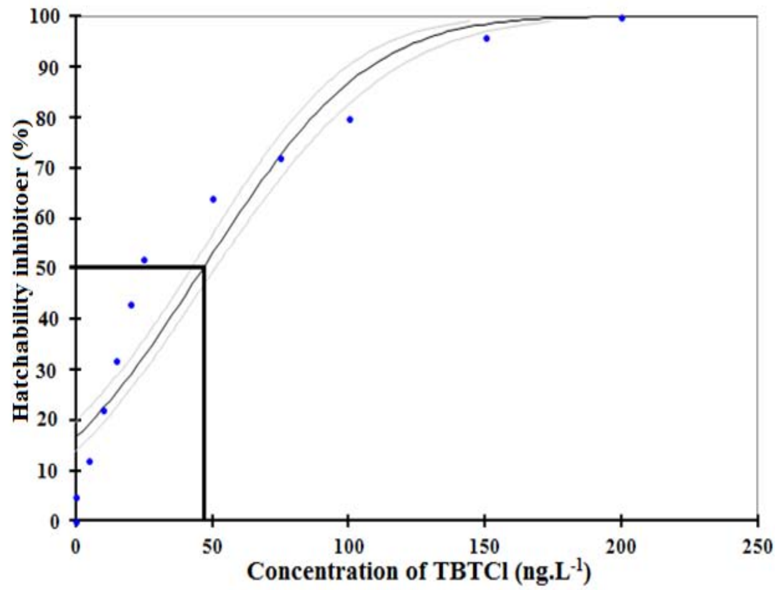

Figure 3: Median Effective Concentration 50% (EC_{50} = 46.48 ngl^{-1}) between TBTCl Concentration and Hatchability Inhibition (%) of *A. salina* Cysts

3. Morphological Effects of *A. salina* Newly Hatching Nauplii after the Exposed the Cysts to Different Concentration of TBTCl

Analysis of the morphological deformities (%) of the means *A. salina* newly hatching nauplii after the exposed the cysts to different concentration of TBTCl as shown in *Table 1*. The higher rate of deformities of newly hatching nauplii at 5 ngl^{-1} TBTCl concentration was 32.00 ± 4.62. Because in this concentration is a chance to newly nauplii stay a longer period survival, which gives greater opportunity to appear changes in shape and deformities. While the lower morphological deformities (%) were1.00 ± 0.00 at concentration 75 ngl^{-1}. Because the chance to survival newly nauplii is very weak and continued growth and development to the body is slowly and it is difficult to note the deformities clearly. As for the other concentration of TBTCl the deformities (%) was between this means. That means the TBTCl different concentration can impact the morphological changes in newly hatching nauplii when exposure the dry cysts to artificial sea water contaminated with TBTCl.

Table 1: Morphological Deformities (%) of Means and SE of Newly Hatching Nauplii *A. salina* Affected by Different Concentration of TBTCl [N: numper of cysts]

TBTCl (ngl^{-1})	N	Deformity (%)
0	300	0.00 ± 0.00[a]
5	300	32.00 ± 4.62[c]
10	300	16.33 ± 3.76[b]
15	300	9.00 ± 0.58[a, b]
20	300	9.67 ± 1.46[a, b]
25	300	6.33 ± 1.76[a, b]
50	300	2.33 ± 0.33[a]
75	300	1.00 ± 0.00[a]

Because in this concentration is a chance to newly nauplii stay a longer period survival, which gives greater opportunity to appear changes in shape and deformities. While the lower morphological deformities (%) were1.00 ± 0.00 at concentration 75 ngl^{-1}. Because the chance to survival newly nauplii is very weak and continued growth and development to the body is slowly and it is difficult to note the deformities clearly. As for the other concentration of TBTCl the deformities (%) was between this means. That means the TBTCl different concentration can impact the morphological changes in newly hatching nauplii when exposure the dry cysts to

artificial sea water contaminated with TBTCl in the low concentrations.

The morphological deformities such as total length in the newly hatching nauplii as can be seen in the increase concentration of TBTCl, the total length of newly hatching nauplii *A. salina* will significantly decrease in general total length in newly hatching nauplii were represented in *Table 2*. This table is shown means of total length newly hatching nauplii shows the control group was (350.9 ± 49.6) μm, but the means for total lengths in different concentration of TBTCl 5,10, 15, 20, 25, 50, 75 ngl^{-1} were (284.6 ± 51.6), (266.8 ± 54.6), (282.2 ± 59.3), (294.9 ± 40.6), (288.8 ± 45.7), (274.8 ± 39.7) and (269.8 ± 54.6) μm, respectively. According to mean and standard error the lowest total length was (266.8 ± 54.6) μm at 10 ngl^{-1} TBTCl concentration compared with the control group.

Table 2: Total Length of Newly Hatched Nauplii After 24hr Exposed to Different Concentration of TBTCl [N: numper of nauplii]

TBTCl (ngl^{-1})	N	Mean ± SEM (μm)	Minimum (μm)	Maximum (μm)
0	35	350.9 ± 49.6^{d}	314.3	451.7
5	35	284.6 ± 51.6^{c}	221.6	377.8
10	35	266.8 ± 54.6^{a,b,c}	202.6	364.2
15	35	282.2 ± 59.3^{a,b}	212.5	398.9
20	35	294.9 ± 40.6^{c}	223.8	389.9
25	35	288.8 ± 45.7^{b,c}	216.5	352.1
50	35	274.8 ± 39.7^{b,c}	244.8	393.3
75	35	269.8 ± 54.6^{a}	201.6	304.2

The morphological changes such as total length and width of body in the newly hatching nauplii as can be seen in *Figures 4, 5, 6 and 7*. In general the regression analysis (*r*) shown the high regression values and this demonstrates a strong inverse relationship between morphological measurements and increase TBTCl concentration. The head width is more affected compared to the total length r = 53 %. And the head is more caricatures and more sensitive to increasing of TBTCl r was 95%. While the abdomen and tail width of body shown moderately affected r = 89 % and decrease when the increasing concentration of TBTCl. In present study need to mention there are not enough studies about effects of TBTCl on the morphological changes in newly hatching nauplii so will be compare these findings with similar studies about nauplii exposed to different types of toxins. For example, Abushaala et al., (2015a) study effect of TBTCl on nauplii stage of *A, salina* and reported in their results the TBTCl had effect the morphology changes of nauplii *A. salina*. On the other hand, (Rao et al., 2007) studies toxicity of Organophosphates on morphology changes in nauplii *A. salina* and significant morphological alteration were noticed in nauplii. In under study Abushaala et al., (2015b) studied effect of Diorun on nauplii stage of *A. salina* their results shown the Diorun had effect the morphology changes in the nauplii stage of *A. salina*. Also Anderson, (2009) his find out about confirmed the impact of alcohol proportion on the improvement rate of *A. salina* nauplii.

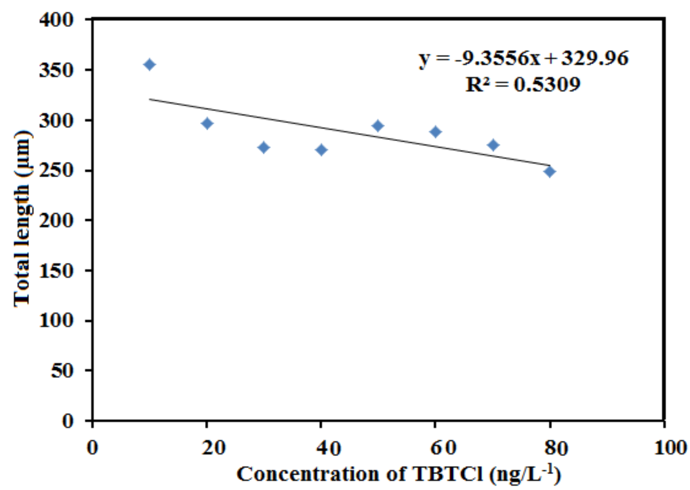

Figure 4: Morophological of Total Length *A. salina* Nauplii after Exposed the Cysts to Different Concentration of TBTCl

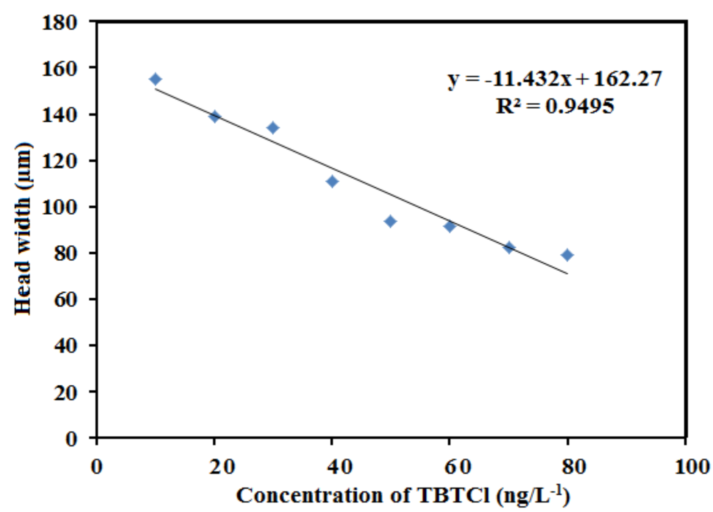

Figure 5: Morphological of Head Width *A. salina* Nauplii after Exposed the Cysts to Different Concentration of TBTCl

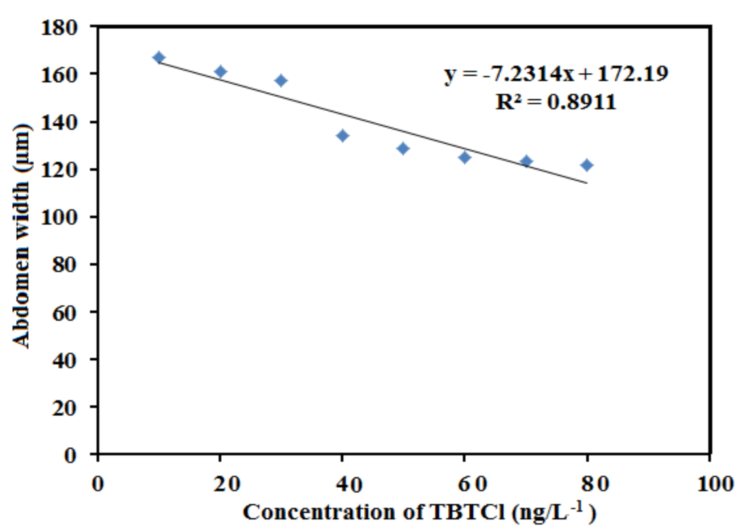

Figure 6: Morphological of Abdomen Width *A. salina* Nauplii after Exposed the Cysts to Different Concentration of TBTCl

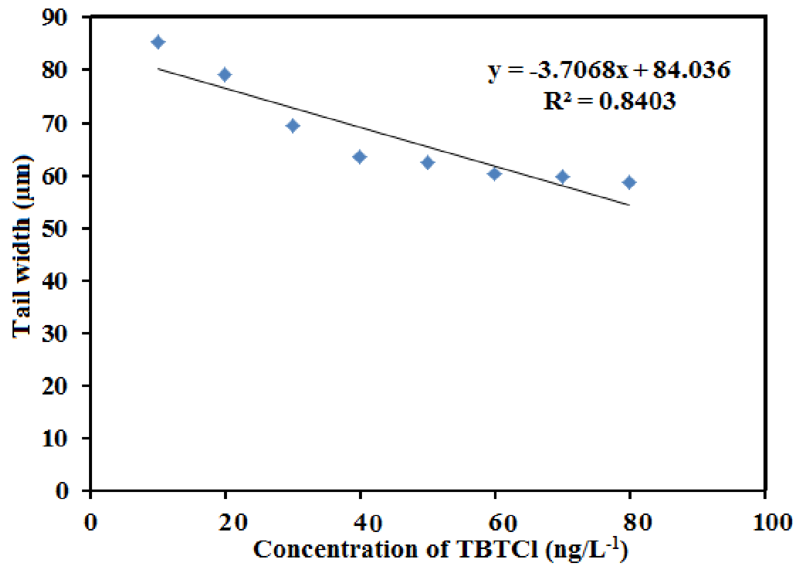

Figure 7: Morphological of Tail Width *A. salina* Nauplii after Exposed the Cysts to Different Concentration of TBTCl

IV. Conclusion

In this study increased TBTCl concentration in solution could significantly decreased the hatchability percentage of *A. salina* cysts and prevented the hatching of larvae. And the EC_{50} value of TBTCl was once recognized as 46.48 ngl^{-1} after 24hr exposure. Early nauplii of *A. salina* is sensitive to TBTCl contamination to reflect hatchablity cysts and early life stage effects of toxicant. In addation, the TBTCl effect on morphological abnormalitiy active newly nauplii *A. salina*. And significant morphological differences were observed in all nauplii exposed to the different concentration of TBTCl are used in this research. These results indicate that in this system TBTCl, it is proven environmentally toxic substances. In general, these results indicated that when increasing TBTCl concentration affected the total body length and the body width of *A. salina* newly hatched nauplii. In spite this result indicated that the system TBTCl is acutely toxic.

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References Références Referencias

1. Abushaala, N. M., Zulkifli, S. Z., Ismail, A., Azmai, M. N. A., & Mohamat-Yusuff, F. (2015a). Selected Morphological Changes in Nauplii of Brine Shrimp (*Artemia salina*) after Tributyltin Chloride (TBTCl) Exposure. *World Applied Sciences Journal, 33*(8), 1334-1340.
2. Abushaala, N. M., Zulkifli, S. Z., Ismail, A., Azmai, M. N. A., & Mohamat-Yusuff, F. (2015b). Lethal concentration 50 (LC_{50}) and effects of Diuron on morphology of brine shrimp *Artemia salina* (Branchiopoda: Anostraca) Nauplii. *Procedia Environmental Sciences, 30*, 279-284.
3. Al-Rashdi, A. (2011). Improving the measurement of butyltin compounds in environmental samples. University of Southampton.
4. Alyŭrŭk, H., & Çavaş, L. (2013). Toxicities of diuron and irgarol on the hatchability and early stage development of *Artemia salina*. *Turkish Journal of Biology, 37*(2), 151-157.
5. Anderson, P. (2009). The Effect of Ethyl Alcohol on the Hatching Success of *Artemia salina*-Winner of the 2009 Robert N. Hancock Memorial Scholarship for the best original technical paper written by a student on a topic in the fields of science or engineering (not peer reviewed). *Journal of the IEST, 52*(1), 9-19.
6. Bartolomé, M. C., & Sánchez-Fortún, S. (2005). Effects of selected biocides used in the disinfection of cooling towers on toxicity and bioaccumulation in *Artemia* larvae. *Environmental Toxicology and Chemistry, 24*(12), 3137-3142.
7. Bellas, J., Ekelund, R., Halldórsson, H. P., Berggren, M., & Granmo, Å. (2007). Monitoring of organic compounds and trace metals during a dredging episode in the Göta Älv Estuary (SW Sweden) using caged mussels. *Water, Air, and Soil Pollution, 181*(1-4), 265-279.

8. Brix, K., Gerdes, R., Adams, W., & Grosell, M. (2006). Effects of copper, cadmium, and zinc on the hatching success of brine shrimp (*Artemia franciscana*). *Archives of environmental contamination and toxicology, 51*(4), 580-583.
9. Caldwell, G. S., Bentley, M. G., & Olive, P. J. (2003). The use of a brine shrimp (*Artemia salina)* bioassay to assess the toxicity of diatom extracts and short chain aldehydes. *Toxicon, 42*(3), 301-306.
10. Castritsi-Catharios, J., Bourdaniotis, N., & Persoone, G. (2007). A new simple method with high precision for determining the toxicity of antifouling paints on brine shrimp larvae (*Artemia*): First results. *Chemosphere, 67*(6), 1127-1132.
11. Fent, K. (1996). Ecotoxicology of organotin compounds. *Critical reviews in toxicology, 26*(1), 3-117.
12. George-Ares, A., Febbo, E. J., Letinski, D. J., Yarusinsky, J., Safadi, R. S., & Aita, A. F. (2003). *Use of Brine Shrimp (Artemia) In Dispersant Toxicity Tests: Some Caveats 1.* Paper presented at the International oil spill conference.
13. Huang, J.-H., Schwesig, D., & Matzner, E. (2004). Organotin compounds in precipitation, fog and soils of a forested ecosystem in Germany. *Environmental Pollution, 130*(2), 177-186.
14. Konstantinou, I., & Albanis, T. (2004). Worldwide occurrence and effects of antifouling paint booster biocides in the aquatic environment: a review. *Environment International, 30*(2), 235-248.
15. Koutsaftis, A., & Aoyama, I. (2008). Toxicity of Diuron and copper pyrithione on the brine shrimp, Artemia franciscana: The effects of temperature and salinity. *Journal of Environmental Science and Health Part A, 43*(14), 1581-1585.
16. Leis, M., Manfra, L., Taddia, L., Chicca, M., Trentini, P., & Savorelli, F. (2014). A comparative toxicity study between an autochthonous *Artemia* and a non native invasive species. *Ecotoxicology, 23*(6), 1143-1145.
17. Libralato, G. (2014). The case of Artemia spp. in nanoecotoxicology. *Marine Environmental Research, 101*, 38-43.
18. Mayorga, P., Pérez, K. R., Cruz, S. M., & Cáceres, A. (2010). Comparison of bioassays using the anostracan crustaceans *Artemia salina* and Thamnocephalus platyurus for plant extract toxicity screening. *Revista Brasileira de Farmacognosia, 20*(6), 897-903.
19. Nunes, B. S., Carvalho, F. D., Guilhermino, L. M., & Van Stappen, G. (2006). Use of the genus Artemia in ecotoxicity testing. *Environmental Pollution, 144*(2), 453-462.
20. Ohji, M., Arai, T., & Miyazaki, N. (2002a). Effects of tributyltin exposure in the embryonic stage on sex ratio and survival rate in the caprellid amphipod Caprella danilevskii. *Marine Ecology Progress Series*, 235, 171-176.
21. Omae, I. (2003). Organotin antifouling paints and their alternatives. *Applied Organometallic Chemistry, 17*(2), 81-105.
22. Rao, J. V., Kavitha, P., Jakka, N., Sridhar, V., & Usman, P. (2007). Toxicity of organophosphates on morphology and locomotor behavior in brine shrimp, *Artemia salina. Archives of environmental contamination and toxicology, 53*(2), 227-232.
23. Revathi, P., & Munuswamy, N. (2010). Effect of tributyltin on the early embryonic development in the freshwater prawn Macrobrachium rosenbergii (De Man). *Chemosphere, 79*(9), 922-927.
24. Rotini, A., Manfra, L., Canepa, S., Tornambè, A., & Migliore, L. (2015). Can *Artemia* Hatching Assay Be a (Sensitive) Alternative Tool to Acute Toxicity Test? *Bulletin of environmental contamination and toxicology, 95*(6), 745-751.
25. Shimasaki, Y., Oshima, Y., Inoue, S., Inoue, Y., Kang, I. J., Nakayama, K., Honjo, T. (2006). Effect of tributyltin on reproduction in Japanese whiting, Sillago japonica. *Marine Environmental Research, 62*, S245-S248.
26. Thomas, K. V., Fileman, T. W., Readman, J. W., & Waldock, M. J. (2001). Antifouling paint booster biocides in the UK coastal environment and potential risks of biological effects. *Marine Pollution Bulletin, 42*(8), 677-688.
27. Voulvoulis, N., Scrimshaw, M. D., & Lester, J. N. (2002). Comparative environmental assessment of biocides used in antifouling paints. *Chemosphere, 47*(7), 789-795.
28. Wang, J., Wang, Q., Li, J., Shen, Q., Wang, F., & Wang, L. (2012). Cadmium induces hydrogen peroxide production and initiates hydrogen peroxide-dependent apoptosis in the gill of freshwater crab, Sinopotamon henanense. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 156*(3), 195-201.

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