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Whole Effluent Toxicity (WET) Test and Toxicity Reduction Evaluation (TRE) of A Meat Processing Factory

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A B S T R A C T

Whole effluent toxicity (WET) test and toxicity reduction evaluation (TRE) of a meat processing factory were performed with *Ceriodaphnia dubia* 3-brood toxicity tests. Results showed that the effluent was chronically toxic to *C. dubia*. It reduced *C. dubia* reproduction by 50% (IC50) at 69.57% effluent. Chloride was suggested as the primary toxic component in the effluent. Other ions such as sulfate and sodium were shown to additively increased chloride toxicity. Results also showed that the effluent was not acutely toxic to *C. dubia*. All or 100% of *C. dubia* were survive during the tests. Effluent dilution with natural water could reduce its toxicity.

Keywords: Whole effluent toxicity (WET) test, Toxicity Reduction Evaluation (TRE), Ceriodaphnia dubia .

1. Introduction

Whole effluent toxicity (WET) describes the aggregate toxic effect of an aqueous sample as measured by an organism's response upon exposure to the sample. WET testing is a vital component in implementing water quality standard under the National Pollutant Discharge Elimination System (NPDES) permits program in accordance with Clean Water Act (CWA. WET test methods include two basic types WET tests, acute and chronic (Grothe, et.al., 1996; U.S. EPA, 2019).

The common components in meat-processing factories are total dissolved solids (TDS), due to the abundant use of salts (i.e., NaCl) during processing. Because of that, some regulatory authorities have set TDS limits for many effluents (Chapman, et.al., 2000). For example, Illinois NPDES sets the limit of 1,000 mg/l TDS for meat processing factories. However, Toxicity related to TDS is mainly due to specific combinations and concentrations of contributing ions such as sodium, potassium, magnesium, chloride, sulfate, nitrate, and bicarbonate. The correlation between increasing TDS concentration and toxicity may vary with different ionic combinations. Therefore, TDS is not the best predictor for toxicity and should not be used as regulatory criteria.

Cations or anions are not present as individual constituents; therefore, TDS effects in the effluents are caused by combinations of ions (Goodfellow, et.al., 2000). Understanding the effects of various ions is fundamental for predicting and characterizing TDS toxicity. For example, Na^+ is not the major contributor to aquatic toxicity, but the associated anion CI^- is more toxic than Na^+ (Mount, et.al., 1997). A variety of approaches can be used to characterize TDS toxicity. For example, conductivity can be used as a general screening tool. According to the United States Environmental Protection Agency (U.S. EPA), when conductivity exceeded 1,000 µmhos/cm, TDS may become a concern for *Ceridaphnia dubia* chronic toxicity (U.S. EPA, 1999). Furthermore, U.S. EPA Phase I Toxicity Identification Evaluation (TIE) can provide useful information. If the results from Phase I TIE of an effluent with high conductivity do not significantly reduce or eliminate toxicity, the concentrations of ions in the effluent may be responsible for the toxicity and should be further evaluated (Goodfellow, et.al., 2000). Using synthetic water, which mimics the major ions in the effluent under evaluation, have also been useful to assess TDS toxicity.

C. *dubia* was chosen as the test species for this study. The choice of the test organism used for the study is based on the consideration importance when measuring WET testing (U.S. EPA, 2019). Several studies have indicated that C. *dubia* and *Daphnia pulex* are the most sensitive test species to sodium chloride (Goodfellow, et.al., 2000). Furthermore, C. *dubia* also were sensitive for many types of pollutants that

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made them the best candidate for toxicity testing and as recommended species in standard methods (U.S. EPA, 1999; U.S. EPA, 2002).

The objective of this study was to measure the toxicity of effluent discharges from a meat packaging industry to C. *dubia* survival and reproduction. To achieve this objective, several effluents and artificial test waters were tested using standard toxicity test procedures (U.S. EPA, 2002). The results of these tests were used to calculate correlations between contributing ions and toxicity. Results of the calculations were then used to predict primary toxicants or ions in the effluent that were responsible for acute and chronic toxicity to C. *dubia.*

2. Materials and Methods

2.1. Test organisms

The test organisms, *Ceriodaphnia dubia*, were obtained from Aquatic Biosystems, Fort Collins, CO, and were cultured according to U.S.EPA methods (U.S. EPA, 2002).

2.2. General water quality

Water samples for each test were monitored for temperature, pH, ammonia, dissolved oxygen (DO), alkalinity, hardness, conductivity, bicarbonate, chlorine, major ions, metals, and total dissolved solids (TDS). Measurements of the first three parameters were conducted with an expandable ion-analyzer (Orion Research Model EA920). DO was measured with an oxygen meter (YSI model 51A). Water hardness and alkalinity were analyzed with the EDTA and the bromocresol green-methyl red titrimetric procedures, respectively (Clesceri, et.al., 1995). Conductivity was measured with a conductivity meter (Amber Science Model 604), while total chlorine concentrations were measured with a DPO colorimeter (Hach Model DR100).

Table 1. Preparation of American Society for Test and Materials (ASTM) water [1)]

Water Type	Reagent Added (mg/l)				Final Water Quality		
	$NaHCO_3$	$CaSO_4.2H_2O$	$MgSO_4$	KCl	pH	Hardness[2)]	Alkalinity[2)]
Mod. Hard	96.0	60.0	60.0	4.0	7-8	80-100	60-70

[1] U.S. EPA, 2002
[2] Express as mg $CaCO_3$/l
Nanopure water was used as solvent, all chemicals were reagent grade

Bicarbonate was calculated from alkalinity according to the equation in the Standard Methods (Clesceri, et.al., 1995). Major cations and metal concentrations were measured using inductively coupled plasma-optical emission Spectroscopy (Varian VISTA-MPX CCD Simultaneously ICP-OES), major anions were measured using ion chromatography (Dionex Model ICS 2500 IonPac AS18 with AG18 guard column). TDS were measured and calculated based on methods published in the Standard Methods (Clesceri, et.al., 1995). A total of 15 ml from a well-mixed sample was filtered through glass fiber filter (PALL, Ann Arbor, MI), and washed with three successive nanopure water rinses. Filtrates were collected in pre-weighed evaporating dishes and then oven dried at 180°C for at least one hour, cooled in a desiccator, and weighed. The cycle of drying, cooling, desiccating, and weighing was repeated until a constant weight was obtained or until weight loss was less than 0.5 mg of previous weight. The TDS (mg/l) then calculated based on the Equation:

$$\frac{(A-B) \times 1000}{\text{sample volume, ml}}$$

where A = weight of dried residue +dish (mg)
B = weight of dish (mg)

2.3. Whole Effluent acute and chronic toxicity tests

The standard method 2002.0 was used for acute toxicity testing with C. *dubia* Roth, et.al., 1989*).* The method measured the acute toxicity of effluents by exposing C. *dubia* to five different concentrations of effluents (6.25%,12.5%, 25%,50%, and 100%) and one standard control in a static non-renewal system for 48hours. Moderately hard reconstituted water known as American Society for Testing and Materials (ASTM) water with hardness of 100mg $CaCO_3$/1 and alkalinity of 60mg $CaCO_3$/1 was used for standard control and dilution water. Preparation and chemical composition of the ASTM water is listed in Table 1. Nanopure water, produced from filtration of distilled water through a Barnstead Nanopure Water Purification System was used to make solutions. These tests were conducted in a controlled environmental room maintained at water temperature of 25°C, and a 16-h light/8-h dark photoperiod. During the light period (16-h), the light was maintained at ambient levels. Neonates less than 24-h of age were used to initiate tests. Six neonates were placed in each chamber. Five replicates *(i.e.,* total 30 organisms) were employed for each test concentration. Test organisms (C. *dubia)* were housed in 30-mlpolystyrenecontainers (Plastic Inc., St. Paul, MN, USA). Food consisted of algae suspension *(Pseudokirchneriella subcapitata)* and yeast-trout chow-cerophyll leaves (YTC) that were given 2 hours before the test. Survival was measured at 24 and 48 hours. Tests were deemed acceptable if control survival was at or above 90% (U.S. EPA, 2002).

The chronic toxicity tests were performed using standard method 1002.0 C. *dubia* survival and reproduction test (U.S. EPA, 2002). This method measured the chronic toxicity of effluents by exposing the test organisms *(i.e.,* C. *dubia)* to five different concentrations of effluents and one standard control as described for the acute test above. These tests were conducted in a controlled environmental room similar to the one used for acute tests. Neonateslessthan24-hof age were used to initiate tests. One neonate was placed in each chamber. Ten replicates *(i.e.,* total of 10 organisms) were

employed for each test concentration. Test organisms (C. *dubia)* were individually housed in 30-mlpolystyrene containers (Plastic Inc., St. Paul, MN, USA). Test solutions were renewed daily. Food consisted of 0.1ml algae suspension *(P. subcapitata)* and 0.1ml yeast-trout chow-cerophyll leaves (YTC) were given daily. Survival and reproduction were measured each day for seven days *(i.e.*, through three-broods). Tests were deemed acceptable if control survival was at or above 80% and the average reproductive output within control groups was minimum 15 neonates (U.S. EPA, 2002).

Ethylenediamine tetraacetate (EDTA) was used during Phase I TIE to evaluate metal toxicity, while sodium thiosulfate was used to evaluate chlorine toxicity. Three different concentrations of EDTA (2, 4, and 6 mg/l) were used for the test based on EPA recommendations (U.S. EPA, 1992). Sodium thiosulfate ($Na_2S_2O_3.5H_2O$) was added at 0.5, 1, and 2 times the theoretical equivalency concentration (TEC) required for chlorine removal (Birge, et.al., 1989). The TEC was calculated to be 2 moles of sodium thiosulfate for 1 mole of chlorine, or 7.0mg/l of sodium thiosulfate hydrate per 1 mg/l chlorine (Birge, et.al., 1989). The amount of sodium thiosulfate added to the effluent was based on chlorine concentrations in the effluent.

In addition to the ASTM water city tap water and receiving waters of a small stream system were also used for the study as comparative controls. Both city tap water and receiving waters were collected as grab samples. The effluent was mimicked using synthetic waters and was then used for characterizing the TDS toxicity. The synthetic waters were prepared to have TDS and chloride concentrations similar to the effluent, which were around 2500 and 500 mg/l respectively. Preparation for synthetic waters is presented in Table 2.

Table 2. Preparation of synthetic test waters. Type A, B, and G used dechlorinated city tap water as the solvent, others used nanopurewater[1)]

Water Type	NaCl	$MgSO_4.7H_2O$	$CaSO_4$	$MgCl_2.6H_2O$	KCl	KNO_3	Na_2SO_4	$NaNO_3$	$CaCO_3$
A	82	-	-	287	-	-	-	-	909
B	247	-	-	573	-	-	-	-	682
C	800	100	500	50	10	200	500	-	-
D	700	500	1000	50	100	200	-	-	-
E	750	200	200	-	100	-	700	500	-
F	700	100	150	50	100	200	1500	-	-
G	-	1000	1000	-	-	-	-	-	-
H	800	1000	1500	-	50	200	-	-	-

[1] mg/l reagent added to the solvent

2.4. Toxicity confirmation with $MgCl_2$

Because chloride concentrations in the effluent were constantly higher than other ionic TDS constituents, toxicity confirmation with $MgCI_2$ was performed to test chloride toxicity at low TDS concentrations. Chloride concentrations were set from 62.50 mg/l to 1,000 mg/l. Standard C. *dubia* chronic toxicity test was performed according to U.S. EPA method mentioned above ASTM water was used as control and dilution water for the test.

2.5. Data analysis

Concentrations of cations and anions resulting in 10% (IC10), 25% (IC25), and 50% (IC50) inhibition of reproduction and resulting in 50% mortality (LC50) were calculated using interpolation *p-percent* inhibition Concentration (ICp) software version 2.0, 1993 (U.S. EPA Environmental Research Laboratory, Duluth, MN). Significant differences in survival between controls and treatment groups were analyzed with Fisher's exact tests (U.S. EPA, 2002), while significant differences in cation and anion concentrations between controls and treatment groups were tested with analysis of variance (ANOVA), followed by the Tukey honest significant difference (HSD) test using Statistica® software (StatSoft, Tulsa, OK, USA). Correlation between cation and anion concentration versus survival and reproduction was calculated using Sigmaplot® Version 8.0 (Systat Software, Point Richmond, CA).

3. Results and Discussion

3.1. Preliminary study

Test results showed that there was some toxicity observed for reproduction (Fig. 1). The IC10, IC25, and median-inhibition concentration (IC50) were calculated at 1.88, 5.09, and 69.57% effluent, respectively (Table 3). Significant *($p<0.05$)* reduction of C. *dubia* reproduction started to appear at 6.25% effluent concentration (Fig. 1). Toxicity identification evaluation (TIE) Phase I study with EDTA and sodium thiosulfate revealed no significant differences between treated and untreated effluent. Based on these preliminary results, the toxicity test for toxicity characterization and comparison among water types would be performed using chronic toxicity procedures.

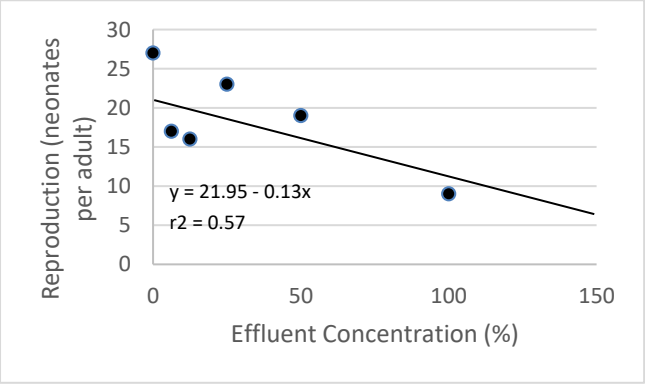

Figure 1. Correlation between meat packaging effluent concentrations and C. *dubia* reproduction determined in whole effluent toxicity (WET) tests. Results are given as means.

Table 3. Comparison of 7-day IC10, IC25, and IC50 between effluent, total dissolved solid (TDS), and chloride on *C. dubia* reproduction

% (IC)	Effluent (%)	TDS (mg/l)	Chloride[1)] (mg/l)	Chloride[2)] (mg/l)
10% (IC10)	1.88	1213.46	95.35	226.87
25% (IC25)	5.09	1328.32	172.37	328.30
50% (IC50)	69.57	1435.81	455.07	444.24

[1] Result from test with synthetic water high TDS concentrations.

[2] Result of confirmation test with $MgCl_2$ with low TDS concentrations.

3.2. Toxicity characterization

The effluent and all water samples affected C. *dubia* reproduction significantly *($p<0.50$).* However, none of them affected survival (Fig. 2). Total dissolved solids (TDS) strongly correlated with C. *dubia* reproduction (Fig.3). Significant reduction of C. *dubia* reproduction began at a TDS concentration of 1394.67 mg/l (Fig. 3). The IC10, IC25, and median-inhibition concentration (lC50) were calculated at 1,213.46, 1,328.32, and, 1,435.81 mg/l TDS, respectively (Table 3). Chloride strongly correlated with C. *dubia* reproduction (Fig.4). The IC10, IC25, and median-inhibition concentration (IC50) were calculated at 95.35, 171.37, and 455.07 mg Cl/l, respectively (Table 3). Sulfate and sodium were unlikely causes of reduction in C. *dubia* reproduction due to weak correlations between the two ions and C. *dubia* reproductive output (Fig. 5 and 6).

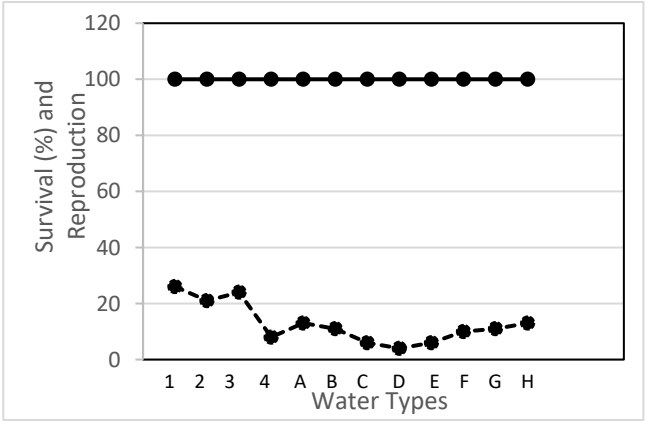

Figure 2. *C. dubia* reproduction (dotted line) and survival (solid line) toxicity test results for controls (water type 1, 2, and 3), effluent (water type 4), and synthetic waters (water types A – H). Results are given as means. Water Type 1 is the standard control using ASTM water with hardness of 100 mg $CaCO_3$/l; type 2 is dechlorinated tap water used as comparative control; type 3 is stream water used as comparative control; types A-H are artificial (synthetic) waters.

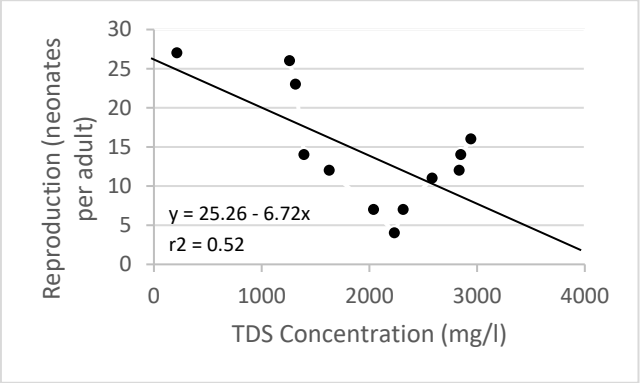

Figure 3. Correlation between total dissolved solid (TDS) and *C. dubia* reproduction. Results are given as means.

3.3. Toxicity confirmation with $MgCl_2$

While the effluent was not acutely toxic to C. *dubia*, it affected C. *dubia* chronically by reducing its neonate production by 50% (IC50) at 69.57% effluent concentration (Table 3). Phase I TIE showed no significant differences between treated and untreated effluent indicating that the toxicity was more likely caused by TDS than other toxicants such as metals or chlorine. Furthermore, high conductivity values (i.e., 3280.71 µmhos/cm) also indicated problems due to TDS toxicity ((U.S. EPA, 1999). Additional tests with synthetic waters showed that TDS concentrations of 1394.6 mg/l and above significantly affected C. *dubia* reproduction (Fig. 3). Strong correlations between TDS and C. *dubia* reproductive output (Fig. 3) gave further indication of TDS role in toxicity. Several studies have documented that TDS was the main cause of toxicity in many effluents (Chapman, et.al., 2000; Goodfellow, et.al., 2000; Mount, et.al., 1997; U.S. EPA, 1999; Kline, et.al., 2000). However, most of them indicated that the primary contributor for TDS toxicity was the combinations and concentration of its ionic constituents (Chapman, et.al., 2000; Mount, et.al., 1997; Kline, et.al., 2000).

The present study indicated that some major ions *(i.e.*, chloride, sulfate, and sodium) were constantly higher in the effluent. These ions were suspected as the primary contributor of TDS toxicity of the effluent. Correlation between major ion concentrations and C. *dubia* reproductive output indicated that chloride was the primary contributor ion of TDS toxicity. It was shown by its stronger correlation (r^2= 0.66) compared to sulfate and sodium with correlation coefficient r^2 of 0.23 and 0.10, respectively (Fig. 4, 5, and 6). Additional tests with synthetic waters showed that chloride concentrations above 500 mg/l significantly reduced C. *dubia* reproduction (Fig. 4).

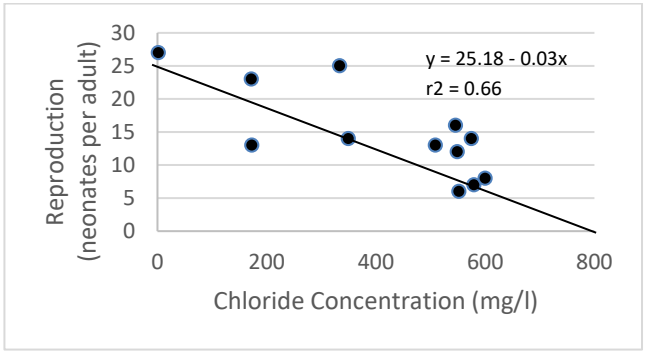

Figure 4. Correlation between chloride concentrations (mg/l) and *C. dubia* reproduction. Chloride concentrations are measured from tests of controls, effluent, and synthetic waters. Results are given as means.

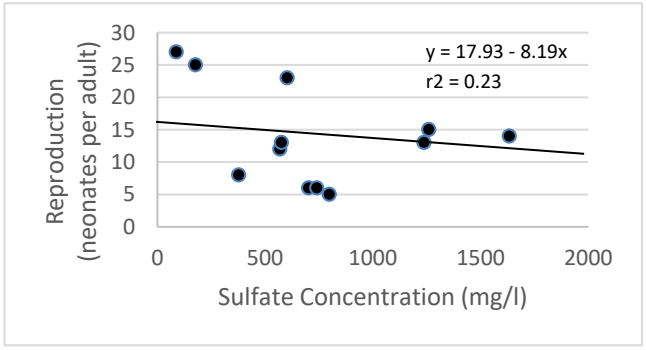

Figure 5. Correlation between sulfate concentrations (mg/l) and *C. dubia* reproduction. Sulfate concentrations are measured from tests of controls, effluent, and synthetic waters. Results are given as means.

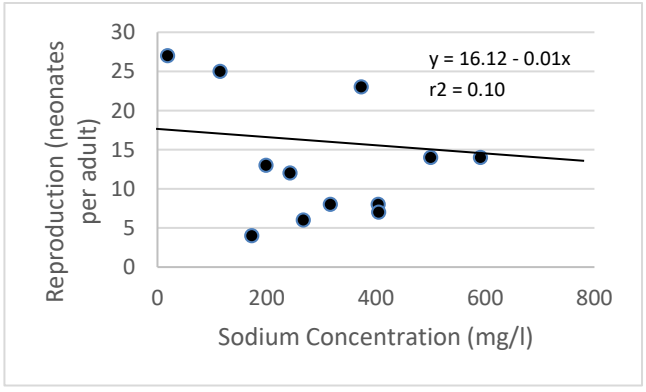

Figure 6. Correlation between sodium concentrations (mg/l) and *C. dubia* reproduction. Sodium concentrations are measured from tests of controls, effluent, and synthetic waters. Results are given as means.

Toxicity confirmation with $MgCl_2$ having low TDS values showed that chloride level at 500 mg/l and above significantly reduced C. *dubia* reproduction with r^2 of 0.92 (Fig.7). The chloride non-observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) to C. *dubia* were 250 and 500 mg/l, respectively while its chronic value (CV) was 353.55 mg/l (Fig.7). The IC10, IC25, and median-inhibition concentration (IC50) were calculated at 226.87, 328.30 and 444.24 mg Cl/l, respectively (Table 3).

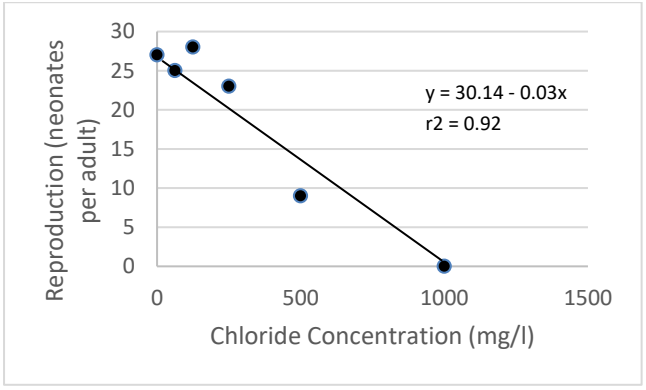

Figure 7. Correlation between chloride concentrations (mg/l) and *C. dubia* reproduction. Chloride concentrations are measured from confirmation tests with $MgCl_2$. Results are given as means.

The results that chloride was toxic to *C. dubia* supported previous studies (Mount, et.al., 1997; Birge, et.al., 1985). Birge et.al. (1985) reported that the non-observed effect concentration (NOEC) and the lowest-observed effect concentration (LOEC) of chloride to *Daphnia pulex* were 314 and 441 mg/l, respectively, and the chronic value (CV) was 372 mg/l. These values were very similar to the NOEC, LOEC, and CV values for *C. dubia* in the present study, which were 250, 500, and 353 mg/l, respectively (Fig. 7). Lower chloride CV value for *C. dubia* compared to *D. pulex* could indicate that *C. dubia* was more sensitive than *D. pulex*. U.S. EPA (1988) adopted the report by Birge et.al. (1985) to be used in National Criteria for chloride, which set a maximum four-day average chloride concentration of 230 mg/l for freshwater ecosystem.

4. Conclusions

The present study demonstrated that effluent from a meat processing industry was chronically toxic to C. *dubia.* There was no evidence was found that the effluent was acutely toxic to C. *dubia.* Additional tests with synthetic waters containing TDS up to 3,000 mg/l observed no acute toxicity to C. *dubia* during 7-day tests. This result suggests that aquatic organisms may tolerate TDS concentrations far above the limit of 1,000 mg/l set in some TDS regulatory permits.

The present study found out, as long as chloride concentrations are low, TDS concentrations as high as 1,314.76 mg/l do not affect C. d*ubia* reproduction. This indicates that TDS is not a good regulatory element for effluent discharging permit. Regulation of the contributing ions in TDS is more important and relevant than TDS alone.

For example, this study demonstrated that media with chloride concentrations above 500 mg/l was chronically toxic to C. *dubia* although TDS concentrations were below 1,000 mg/l. Therefore, chloride may be a better indicator used for a regulatory element of effluent than the TDS value alone.

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