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Impact of Copper and Cadmium on the Nutritional Value of the Rotifer *Brachionus plicatilis* and their Effect on *Dicentrarchus labrax* Fish Larvae.

Heba S. El-sayed[1*] and Gihan M. El-khodary[2]

1- Fish Reproduction and Larval Rearing Lab. (Marine Hatchery), Aquaculture Division, National Institute of Oceanography and Fisheries, Alexandria, Egypt.
2- Zoology Departments, Faculty of Science, Damanhour University, Egypt.

***Corresponding author:** hebasaad2222@yahoo.com

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ABSTRACT

Brachionus plicatilis is a rotifer that considered an important live feed in aquaculture for feeding the initial stages of many fish larvae. This study aimed to determine the growth, survival rate and biochemical alterations of *Dicentrarchus labrax* (sea bass) larvae fed on *B. plicatilis* treated with sublethal concentrations of cadmium chloride ($CdCl_2$) and copper sulphate ($CuSO_4$). This study is extended to evaluate the alteration in the nutritional value of *B. plicatilis* upon the exposure to sublethal doses of these heavy metals. The results showed that the total length and width means of *D. labrax* larvae reared on *B. plicatilis* exposed to $CuSO_4$ and $CdCl_2$ increased significantly when compared to their control ($p< 0.05$.). *D. labrax* larvae fed on rotifers treated with $CuSO_4$ and $CdCl_2$ showed a significant decrease in their survival percentages ($p < 0.001$) and their total lipids and protein levels ($p < 0.001$) when compared to their control. Moreover, the level of carbohydrate and lipid decreased significantly ($p < 0.05$) in the *B. plicatilis* exposed to sublethal concentration of $CdCl_2$ and $CuSO_4$. Most of the essential and nonessential amino acids in *B. plicatilis* exposed to these heavy metals were partially decreased while methionine, phenylalanine, histidine and leucine showed significant increase. Treating *B. plicatilis* with $CdCl_2$ showed a significant decrease in mono and poly unsaturated fatty acids. The oleic acid ((C20:1 ω9) and the caprylic acid (C8:0) decreased in *B. plicatilis* exposed to $CdCl_2$ and $CuSO_4$, while the Eicosatrienoic acid (C 20:3ω3) and the Eicosapentaenoic acid (C 20:5ω3) were not detected. The Arachidonic acid (C20:4 ω6) decreased significantly in treated *B. plicatilis* with $CdCl_2$. The present work concluded that, *D. labrax* larvae feeds on *B. plicatilis* exposed to sublethal concentration of Cd and Cu heavy metals showed marked effect on the survival rate and biochemical composition.

INTRODUCTION

Dicentrarchus labrax (sea bass) is one of the most important commercial fish species in Egypt, and it is reared in marine and brackish water (El-Shebly, 2009). Marine sea bass fry suffers from increasing death during the early rearing time in hatcheries. This may be due to different factors such as availability and nutrition of live food supplemented to the larvae at different stages of their growth (Zaki and Saad, 2010). The rotifer *Brachionus plicatilis* is indispensable for aquaculture since it

is widely used as a primary food organism for the initial stages of many fish and crustacean larvae (Ando *et al*., 2004; Cheng *et al*., 2004).

The nutritional qualities of the rotifers are important for the optimal growth and survival of fish larvae**.** The effects of rotifer on larval growth and survival are reported by Olsen *et al*. (1993) and Rainuzzo *et al*. (1997).

Heavy metals are widely recognized as potential toxic agents to zooplankton (Gama-Flores *et al*., 2006). These metals are non-biodegradable and can be accumulated by organisms to a level that affects their physiological states (El-khodary and El-Sayed, 2011). Copper (Cu) is an essential metal for the function of most living organism. It is a vital component as a co-factor of enzymes as well as respiratory pigments (Vance and Vance 2002). In contrast, cadmium (Cd) has no known biological role and exhibits high toxicity for living organisms (Gupta, 2013). Cd ions interact with various cell structures causing harmful biochemical shifts and inhibiting several enzymes activity where it causes a damage of cell membrane structure and affects its functions (Viarengo, 1994).

Amino acids are the building blocks for protein synthesis. They are important energy substrates and are involved in specific physiological functions (Guoyao *et al.*, 2014). Lipids are considered that main sources of energy for pre-feeding fish larval stages (Evans *et al*., 2000). Essential fatty acids such as eicosapentaenoic acid and arachidonic acid are essential nutrients for fish larvae (McEvoy *et al*., 1998; Estevez *et al*., 1999; Zaki and Saad, 2010; Costa *et al*., 2015). Recently, there has been an upsurge of research on the beneficial effects of omega-3 fatty acids on health and disease (De Camargo Talon *et al*., 2015; Drudi *et al*., 2017). The present study investigates the morphological changes, growth, and survival rates of *Dicentrarchus labrax* larvae fed on *Brachionus plicatilis* treated with sublethal dose of Cu, Cd. Further study is extended to evaluate the alterations in biochemical composition of rotifers upon the treatment of *B. plicatilis* with sublethal concentrations of $CdCl_2$ and $CuSO_4$.

MATERIALS AND METHODS

Culture of *B. plicatilis*

B. plicatilis obtained from fish reproduction and spawning Lab. (Marine hatchery) located in National Institute of Oceanography and Fisheries, Alexandria, Egypt. Filtered sea water was used for the culture. Investigated samples were fed on *Nannochloropsis salina* at a density 10×10^6 cells/ ml. The temperature of the culture of rotifers was maintained at 27 ^{o}c and the salinity was held at $24gL^{-1}$.

Culture of *D. labrax* larvae

Larvae obtained from eggs derived from induced spawning of *D. labrax* broad stock kept at the marine hatchery of National Institute of Oceanography and Fisheries, Alexandria, Egypt.

Effect of chronic exposure to Cu and Cd on nutritional value of *B. plicatilis*

Based on LC_{50} values determined by El-khodary and El-Sayed (2011), *B. plicatilis* was exposed to sublethal concentrations of $CdCl_2$ and $CuSO_4$ at 0.3 mg/l and 0.25 mg/l, respectively for 6 days.

Feeding regime schedule

The feeding schedule for the *D. labrax* larvae was performed by adding enriched *B. plicatilis* in density of 10-35 individuals/ml according to grading age. Upon having *Artemia* nauplii and metanauplii (normal untreated) as second food sources for larvae with increasing mouth opening and age from 15-45-day post hatch

(dph) and the number of rotifers decreased to 20 ind/ml in the feeding regime according to FAO(1999).

Laboratory investigations

Morphometric measurements and survival rates of *D. labrax* larvae reared on *B. plicatilis* (normal, and treated with Cu and Cd)

Twenty larvae of *D. labrax* per tank were taken at day 5 after hatching. Nine tanks, each 100 L as triplicate, were used for the experiment (39 gL^{-1} water salinity, pH 8 and temperature 16 ± 1°C). The tanks were cleaned daily, as well as removing about 70% of their water and the dead larvae.

The morphometric measurement, and survival rates were monitored at days 8, 10, 15, 20, 25, 30, 35, 40 and 45 after the larvae reared on *B. plicatilis* treated with Cu and Cd compared with control. The growth measurements of *D. labrax* larvae include total length (L) and width (W) to the nearest millimeters (mm), and survival rates ((No. of fish larvae at end / No. of fish larvae at the start) ×100) were determined.

Biochemical analysis

Total protein, carbohydrate and lipid contents of *B. plicatilis* and *D. labrax* larvae were determined according to Lowery *et al.* (1951), Dubois *et al.* (1956), and Folch *et al.,* (1957) respectively.

Amino acid analysis

The dried samples of *B. plicatilis* were put in diethyl ether for 24 hours to remove lipid and were then dried. Amino acids were analyzed by sealed tube hydrolysis with 6N HCL for 22 hours at 110^{o}C. After hydrolysis, the acid was evaporated in vacuum oven. The residue of the sample was dissolved in 1 ml of sample dilution (diluting buffer) (0.2 M, pH 2.2) to complete the sample dissolving. Automatic amino acid analyzer was used for amino acid determination (Dionex ICS-3000) (Block *et al*., 1948).

Fatty acid analysis

Fatty acids were methylated with Boron Trifluoride (BF_3) in methanol. Fatty acids methyl esters (FAMEs) were obtained by the method according to Metcalfe *et al.* (1966). The resulting methyl esters were then analysed using an Agilent Gas Chromatograph system according to Radwan (1978).

Statistical analysis

All experiments were conducted three times. Data were represented as mean ± SD. Analysis by one-way of variance (ANOVA) was used, significant differences were considered when $p < 0.05$.

RESULTS

Morphometric measurements, and survival rates of *D. labrax* larvae reared on *B. plicatilis* treated with $CdCl_2$ and $CuSO_4$

The total length and width means of *D. labrax* larvae reared on *B. plicatilis* treated with $CdCl_2$ and $CuSO_4$ during the period of 5-45 dph were showed in Figures 1 and 2 respectively. On one hand, the mean total length of larvae enriched with non-treated and treated *B. plicatilis* with $CuSO_4$ decreased significantly from 5.2 ± 0.25 to 4.6 ± 0.12 mm at 10 dph, and from 5.6 ± 0.36 to 4.8 ± 0.28 mm at 15 dph, while it showed significant increase ($p \leq 0.05$) at 30, 35 and 40dph. At 40 dph the mean total length of *D. labrax* larvae enriched with non-treated and treated *B. plicatilis* with $CuSO_4$ reached 9.8 ± 0.23 and 12.1 ± 0.28 mm respectively. On the other hand, the total length of larvae reared on *B. plicatilis* treated with $CdCl_2$ showed significant

decrease at 8 and 10 dhp. (3.4 ± 0.32 mm and 4 ± 1 mm respectively), and significant increase at 30 dph (8.3 ± 0.57 mm). The mean width of *D. labrax* larvae reared on *B. plicatilis* treated with $CuSO_4$ significant decrease at 8, 10 and 15 dph, and significant increase at 20, 30 and 40 dph. The width of larvae reared on *B. plicatilis* treated with $CdCl_2$ showed significant decrease at 15 dph, and significant increase at 20, 30 and 40 dph (Figure 2).

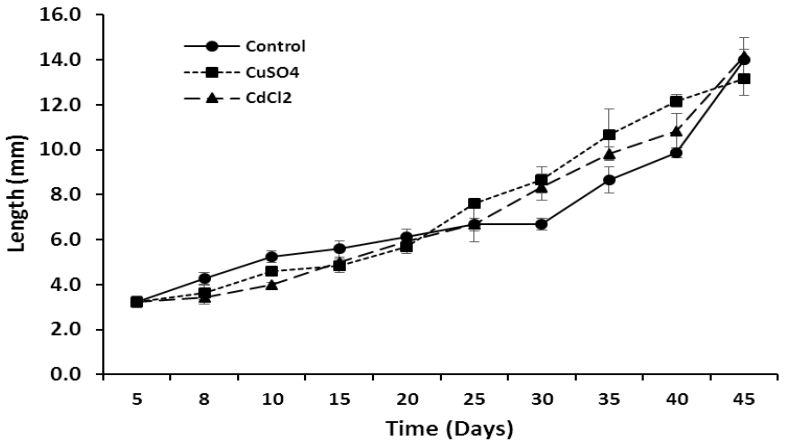

Fig. 1: Total length (mm) of *D. labrax* larvae reared on *B. plicatilis* treated with sublethal concentrations of $CuSO_4$ and $CdCl_2$.

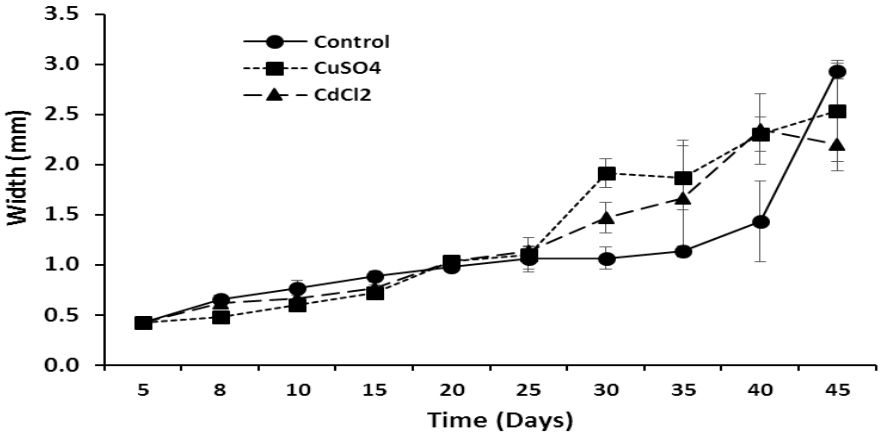

Fig. 2: Width of *D. labrax* larvae reared on *B. plicatilis* treated with sublethal concentrations of $CuSO_4$ and $CdCl_2$.

Evaluation of the morphological changes of *B. plicatilis* and larval performance of sea bass *D. labrax* enriched on treated *B. plicatilis* was shown in figures 3, 4. B. *plicatilis* treated with $CuSO_4$ showed a larger size than their normal control (Figure 3C), while the *D. labrax* larva (12 dph) enriched on treated *B. plicatilis* with $CuSO_4$ exhibited deformed vertebral column (Figure 4C).

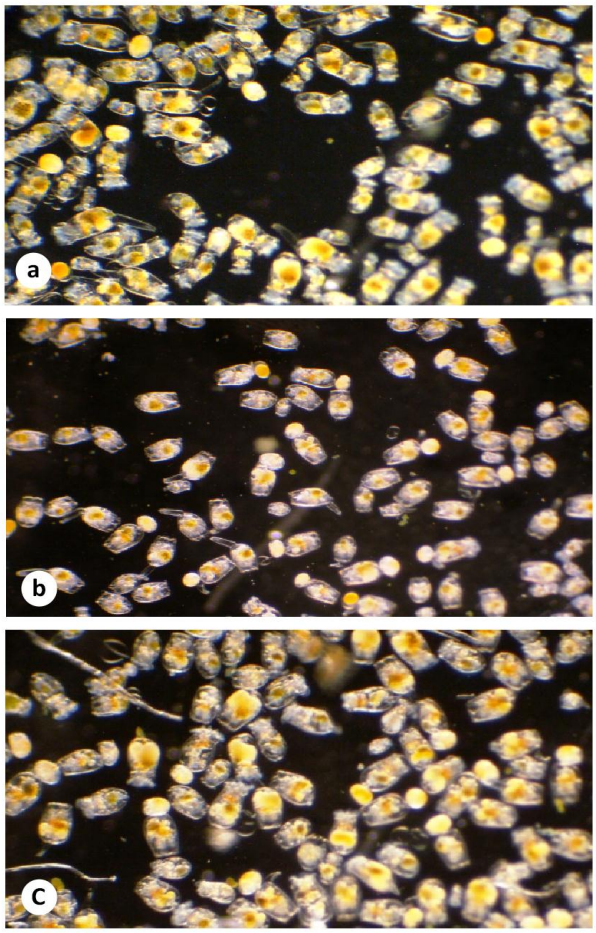

Fig. 3: Shows the Rotifers under different treatment settings. Control Rotifers (A) (Mag45X). Rotifers treated with $CdCl_2$ sublethal dose (B) (Mag35X). Rotifers treated with CuSo4 sublethal dose (C) (Mag35X).

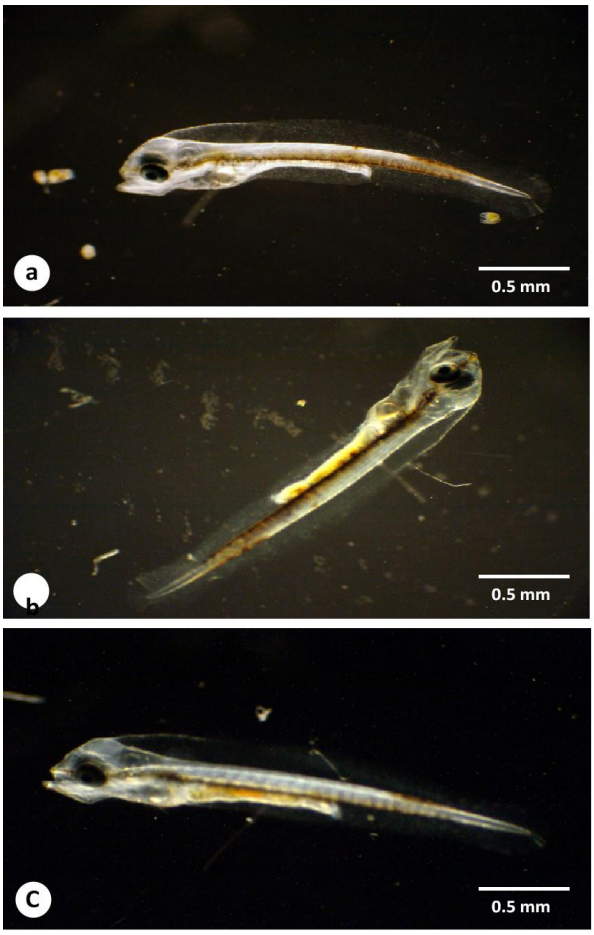

Fig. 4: Sea bass post larva after feeding with rotifers. Full healthy containing digested rotifer inside gut (A) 12dph with Mag 45X. Sea bass post larva fed with rotifers exposed to $CuSO_4$ sublethal concentration (B) (Mag35X) 12dph. Sea bass post larva fed with rotifers exposed to $CdCl_2$ sublethal concentration (C) (Mag35X)12dph.

The survival percentages of *D. labrax* larvae reared on *B. plicatilis* during the period of 5-45 dph are represented in figure 5. The results indicated that the survival percentages of *D. labrax* larvae were significantly decreased ($p \leq 0.001$) starting at 8 days of hatching larvae after reared on *B. plicalits* treated with $CdCl_2$ and $CuSO_4$ (Figure 5).

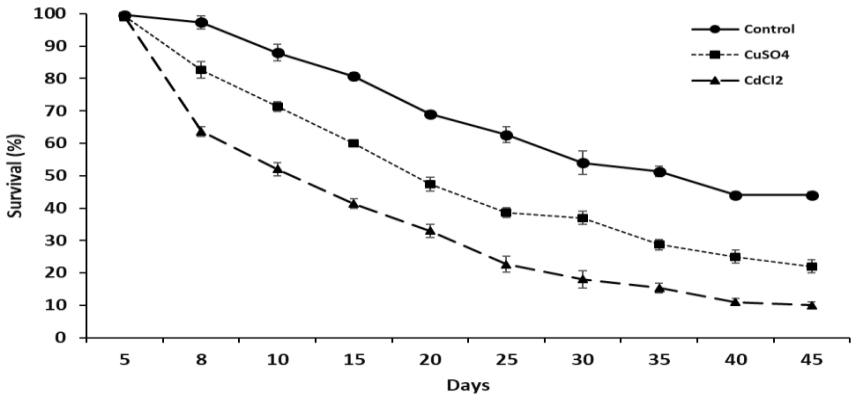

Fig. 5: Survival percentages of *D. labrax* larvae reared on *B. plicatilis* treated with sublethal concentrations of $CuSO_4$ and $CdCl_2$.

The total protein, carbohydrates, and lipids contents of *D. labrax* larvae and *B. plicatilis* exposed to sublethal concentrations of $CdCl_2$ and $CuSO_4$

The Biochemical compositions of *D. labrax* larvae reared on the non-treated and treated *B. plicatilis* with sublethal concentrations of $CdCl_2$ and $CuSO_4$ were shown in figure 6 A, B and C. The data showed that a highly significant decrease in the total lipid and protein levels of *D. labrax* larvae reared on *B. plicatilis* exposed to sub-lethal concentration of $CuSO_4$ and $CdCl_2$ ($p < 0.001$). The carbohydrate contents were the same when treated and non-treated groups were compared (Figure 6 B). The biochemical compositions of *B. plicatilis* after exposure to sublethal concentrations of $CdCl_2$ and $CuSO_4$ are shown in Figure 7 A, B and C. The results revealed that the protein content of the *B. plicatilis* exposed to sublethal concentration of $CdCl_2$ significantly decreased (Figure 7 A). The carbohydrate and lipid contents showed significant decrease ($p < 0.05$) in the *B. plicatilis* treated with $CdCl_2$ and $CuSO_4$ when compared to their control (Figure 7 B and C).

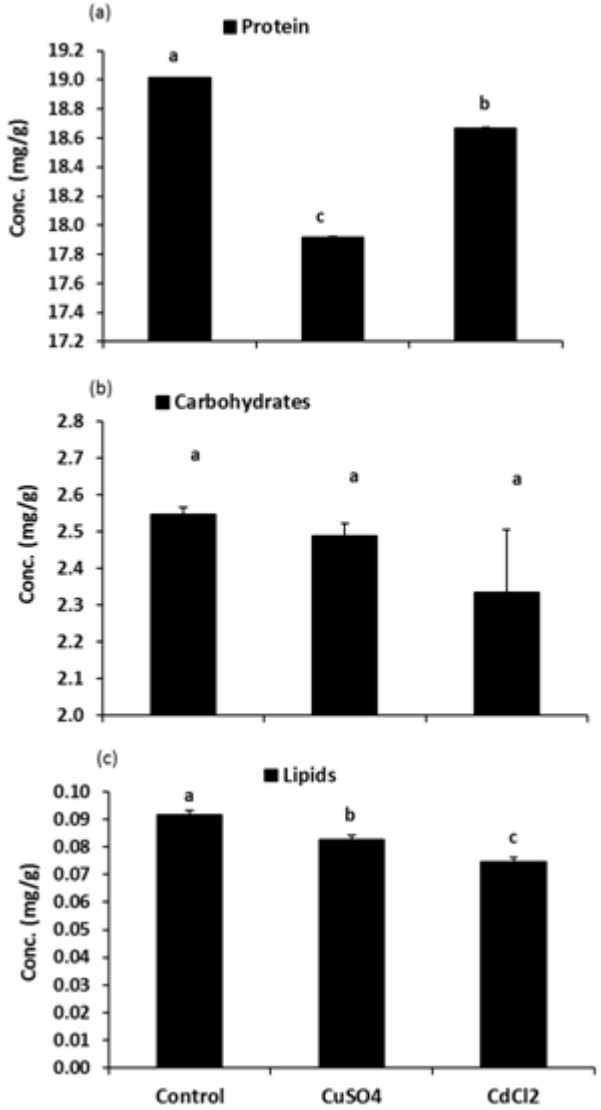

Fig. 6: Total protein, carbohydrates and lipids content in *D. labrax* larvae reared on *B. plicatilis* treated with sublethal concentrations of $CuSO_4$ and $CdCl_2$.

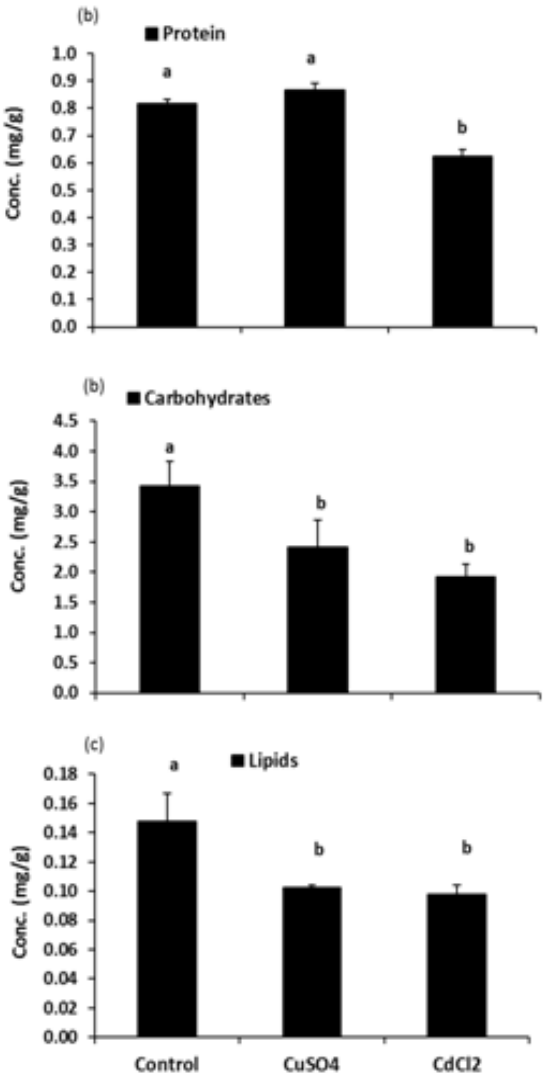

Fig. 7: Total protein, carbohydrates and lipids content in *B. plicatilis* treated with sublethal concentrations of $CuSO_4$ and $CdCl_2$.

The amino acid contents of *B. plicatilis* after exposure to sublethal concentrations of $CdCl_2$ and $CuSO_4$

The amino acid contents of *B. plicatilis* after exposure to sublethal concentrations of $CdCl_2$ and $CuSO_4$ are shown in Table 1. There are about 13 different amino acids in the tissues of non-treated *B. plicatilis*, including 7 essential and 6 nonessential amino acids. The most abundant essential amino acids are Arginine followed by Lysine. While glutamic and Cystine are the most abundant non-essential amino acids. The results showed that there is a great reduction in Arginine and Lysine in CdCl2 and CuSo4 treated B. plicatilis while the remaining essential amino acids showed significant increase. On other side all nonessential amino acids showed significant increase in the treated groups

Table 1: Amino acid analysis (mg/g) of the investigated *B. plicatilis* (Mean ± SD)

Essential amino acid (mg/g)	**Non – treated *B. plicatilis***	**Treated *B. plicatilis* with CdCl2**	**Treated *B. plicatilis* with CuSo4**
Lysine	8.426 ± 0.046 [a]	0.825 ± 0.035 [b]	0.596 ± 0.043 [c]
Threonine	0.571 ± 0.021 [a]	N.D.	0.459 ± 0.025 [b]
Methionine	0.713 ± 0.028 [c]	10.015 ± 0.032 [b]	10.278 ± 0.034 [a]
Phenyl alanine	0.305 ± 0.036 [c]	3.453 ± 0.029 [a]	2.429 ± 0.016 [b]
Histidine	0.284 ± 0.062 [c]	1.634 ± 0.041 [b]	2.261 ± 0.019 [a]
Leucine	0.481 ± 0.031 [c]	0.550 ± 0.016 [b]	0.641± 0.04 [a]
Arginine	24.8 ± 0.04 [a]	1.282 ±0.053 [b]	1.083 ± 0.029 [c]
Non - Essential amino acid (mg/g)	**Non – treated *B. plicatilis***	**Treated *B. plicatilis* with CdCl2**	**Treated *B. plicatilis* with CuSo4**
Alanine	1.893 ± 0.013 [a]	0.763 ± 0 .034 [c]	0.836 ± 0.019 [b]
Glycine	0.140 ± 0.015	N.D.	N.D.
Serine	0.0894 ± 0.021	N.D.	N.D.
Glutamic acid	9.367 ± 0.031 [a]	2.759 ± 0.04 [b]	1.114 ± 0.023 [c]
Aspartic acid	0.517 ± 0.05 [b]	0.484 ± 0.017 [c]	1.295 ± 0.039 [a]
Cystine	8.719 ± 0.027 [a]	2.734 ± 0.02 [b]	1.647 ± 0.026 [c]

Fatty acid profiles of non-treated and treated *B. plicatilis* with sublethal concentration of $CdCl_2$ and $CuSO_4$

The profiles of fatty acids detected through GC analysis from non-treated and treated *B. plicatilis* with exposure to sublethal doses of $CdCl_2$ and $CuSO_4$ were shown in Table 2. In non-treated *B. plicatilis*, 27 different fatty acids were detected including 14 unsaturated fatty acids and 13 saturated fatty acids. The results showed that caprylic acid (C8:0) and the palmitic acid (C16:0) were the most abundant SFAs in non-treated *B. plicatilis*. The caprylic acid showed a significant decrease, whereas the palmitic acid showed a significant increase in *B. plicatilis* exposed to $CdCl_2$ and $CuSO_4$. The major acids detected among the MUFAs included Erucic acid (C22: 1 ω9), Elaidic acid (oleic acid) (C18:1 ω9) and Elcosenoic acid (C20:1 ω9). The oleic acid was in a significant decrease in *B. plicatilis* exposed to $CdCl_2$ and $CuSO_4$, while the major abundant PUFAs were Eicosadienoic acid (C20:2 ω6), Arachidonic acid Omega 6 (C20:4) and Eicosapentaenoic acid Omega 3 (C20:5) (Table 2). Eicosatrienoic acid (C 20:3ω3) and Eicosapentaenoic acid (C 20:5ω3) were non-detected, while Arachidonic acid (C20:4 ω6) decreased significantly in treated *B. plicatilis* with $CdCl_2$. The results also showed that the total fatty acid contents of *B. plicatilis* treated with $CdCl_2$ significantly decreased when compared to non-treated control (Table 2). As a total, the total number of saturated fatty acids showed

significant increase in treated groups. While both mono-saturated and polyunsaturated fatty acids showed significant decrease.

Table 2: Fatty acid profiles (%) of the investigated *B. plicatilis* (Mean ± standard deviation)

No	Fatty acids / Saturated fatty acids (SFAs)	Carbon atom(n)	Non -treated *B. plicatilis*	Treated *B. plicatilis* with CdCl2	Treated *B. plicatilis* with CuSo4
1	Caproic acid	C6:0	0.541 ± 0.010^{b}	0.09 ± 0.021^{c}	1.113 ± 0.015^{a}
2	Caprylic acid	C8:0	7.03 ± 0. 02^{a}	0.010 ± 0.006 ^{c}	1.72 ± 0.020 ^{b}
3	Capric acid	C10:0	0.180 ± 0.026^{b}	0.100 ± 0.026^{b}	0.353 ± 0.025^{a}
4	Undecylic acid	C11:0	1.593 ± 0.040 ^{b}	0.750 ± 0.015^{c}	3.240 ± 0.36^{a}
5	Lauric acid	C12:0	0.180 ± 0.026^{b}	0.390 ± 0.021^{a}	0.187 ± 0.025^{b}
6	Tridecylic acid	C13:0	2.423 ± 0.025^{a}	0.770 ± 0.025 ^{b}	0.251 ± 0.20^{c}
7	Myristic acid	C14:0	0.203 ± 0.025^{c}	0.950 ± 0.025 ^{b}	3.473 ± 0.038^{a}
8	Pentadecylic acid	C15:0	0.253 ± 0.025^{b}	0.500 ± 0.026 ^{a}	0.113 ± 0.015^{c}
9	Palmitic acid	C16:0	6.470 ± 0.020^{c}	18.930 ± 0.20 ^{a}	12.357 ± 0.25^{b}
10	Margaric acid	C17:0	0.073 ± 0.020^{c}	2.590 ± 0.12 ^{a}	0.140 ± 0.020^{b}
11	Stearic acid	C18:0	1.173 ± 0.021^{c}	2.190 ± 0.14 ^{b}	2.370 ± 0.020 ^{a}
12	Arachidic acid	C20:0	1.283 ± 0.021^{a}	N.D.	1.163 ± 0.015^{b}
13	Heneicosylic acid	C21:0	0.770 ± 0.020^{a}	N.D.	0.613 ± 0.015^{b}
Total			**22.173 ± 0.176^{b}**	**27.270 ± 0.050^{a}**	**27.363 ± 0.115^{a}**
Monounsaturated fatty acid					
14	Teradecanoic acid	C14:1	0.140 ± 0.01^{b}	0.357 ± 0.015^{a}	0.133 ± 0.015^{b}
15	14,Pentadecanonic acid	C15:1	0.087 ± 0.025 ^{c}	0.527 ± 0.021 ^{a}	0.158 ± 0.02 ^{b}
16	9Hexadecenoic acid	C16:1	0.523 ± 0.025 ^{c}	1.257 ± 0.021 ^{a}	1.037 ± 0.015 ^{b}
17	Heptadecenoic acid	C17:1	0.171 ± 0.02 ^{c}	1.011 ± 0.02 ^{a}	0.58 ± 0.01 ^{b}
18	Elaidic acid (oleic acid) omega 9	C18:1	4.373 ± 0.2 ^{a}	0.947 ± 0.015 ^{b}	0.573 ± 0.02 ^{c}
19	Elcosenoic acid omega 9	C20:1	3.767 ± 0.15 ^{b}	1.181 ± 0.027 ^{c}	5.81 ± 0.36 ^{a}
20	Erucic acid Omega 9	C22:1	5.696 ± 0.091 ^{a}	2.268 ± 0.17 ^{c}	5.497 ± 0.42 ^{b}
Total			**14.757 ± 0.112^{a}**	**7.547 ± 0.01^{c}**	**13.790 ± 0.010^{b}**
Polyunsaturated fatty acid					
21	Linolenic acid omega 3	C18:3	0.6 ± 0.02^{a}	0.480 ± 0.026^{b}	N.D.
22	Linoleic acid Omega 6	C18:2	3.07 ± 0.15^{a}	1.272 ± 0.02^{b}	N.D.
23	Eicosapentaenoic acid Omega 3	C20:5	3.88 ± 0.025^{a}	N.D.	3.55 ± 0.01^{b}
24	Arachidonic acid Omega 6	C20:4	4.74 ± 0.12^{b}	0.553 ± 0.002^{c}	5.234 ± 0.015^{a}
25	Eicosatrienoic acid Omega 3	C20:3	2.72 ± 0.07^{b}	N.D.	2.532 ± 0.02^{a}
26	Eicosadienoic acid Omega 6	C20:2	5.476 ± 0.03^{a}	1.025 ± 0.021^{c}	5.011 ± 0.01^{b}
27	Docosadienoic acid Omega 6	C22:2	0.102± 0.02^{b}	0.087 ± 0.015^{b}	0.24 ± 0.01^{a}
Total			**20.58 ± 0.1^{a}**	**2.92 ± 0.05^{c}**	**16.57 ± 0.01^{b}**
	Total fatty acid		**57.85 ± 0.115^{a}**	**37.68 ± 0.095^{b}**	**57.51 ± 0.38^{a}**

DISCUSSION

Branchionus plicatilis is one of the most known forms of all rotifers which serve as an ideal starter diet for early larval stages of many fish. The current study evaluated the impact of feeding with *B. plicatilis* exposed to sublethal concentrations of $CdCl_2$ and $CuSO_4$ on the morphometric changes, survival, and some biochemical alterations of *D. labrax* larvae. Heavy metals are widely known as a potential toxic agent to zooplankton and other living organisms (Gama-Flores *et al*., 2006; Fokina *et*

al., 2013; Wang *et al*., 2014). Hwang *et al*. (2016) reported that Cu and Cd have a toxic effect on *B. plicatilis*. Consistent with the previous finding, the present results showed that the treatment with sublethal concentrations of $CdCl_2$ and $CuSO_4$ decreased the total mean length and width of *D. labrax* larvae during the period of 5-15dph when compared with non-treated control. We have found that the survival percentage of *D. labrax* larvae treated with *B. plicatilis* exposed to sublethal concentrations of $CdCl_2$ and $CuSO_4$ decreased when compared to *D. labrax* larvae fed on non-treated *B. plicatilis.* This finding confirmed that the toxicity of $CdCl_2$ and $CuSO_4$ on *D. labrax* larvae by indirect way upon rearing of fish larvae on treated rotifers with these heavy metals.

Growth reduction in both Cd-exposed and Cu-exposed fish may result from impaired perception and reduced food uptake. Cd and Cu significantly reduced embryonic survival and quality of newly hatched fish larvae (Witeska *et al*., 2010; Ługowska and Kubik, 2011). However, many studies showed higher toxicity of Cu compared to Cd (Jezierska and Witeska 2001; Zhu *et al*. 2011). Furthermore, some studies indicated that Cd is more toxic to initial stages of some fish species than Cu (Jezierska *et al*. 2009a, b; Barjhoux *et al.*, 2012). According to the present data, *D. labrax* larvae reared on *B. plicatilis* treated with Cd increased the rate of larval mortality when compared to *D. labrax* larvae reared on *B. plicatilis* treated with Cu. In the present study, the total mean length and width of *D. labrax* reared on treated *B. plicatilis* increased significantly at 30, 35 and 40 dph. This may be due to synthesis of metallotionine. Yves *et al.* (1993) showed that the aquatic organisms synthesize metallothioine as a defense against toxic metal influence in laboratory trials where exposure conditions can differ greatly from the natural environment. Developmental abnormalities may also result from Cd and Cu toxicity (Ozkan *et al*. 2011).

Toxicity with Cd and Cu may have impact on biochemical constituents such as glycogen, total proteins, lipid, and free amino acids (Sobha *et al*., 2007). In the present study, we have found that a significant decrease in the total protein and lipids in *D. labrax* larvae reared on *B. plicatilis* treated with $CdCl_2$ and $CuSO_4$ could be due to dysfunction of the metabolism inside the body due to the toxic effect of these heavy metals. Such finding agrees with the previous finding by Fokina *et al.* (2013). Only exposure of *B. plicatilis* to $CdCl_2$ led to a significant decrease in the protein content which may explain why $CdCl_2$ was more toxic than $CuSO_4$ on *B. plicatilis.* The content of both carbohydrates and lipids showed a significant decrease in *B. plicatilis* treated with $CuSO_4$ and $CdCl_2$. Sekar *et al*. (2009) and Sreenivasan *et al.* (2009) suggested that the decrease in the protein content in different tissues of *Spiralothelphusa hydrodroma* after exposure to sublethal concentrations of textile industry dye effluent and cypermthrin may be due to an increased proteolysis and the possible utilization of the products and their degradation in the metabolic process. In addition, they indicated that the decrease in the carbohydrate content was due to glycogenolysis and the utilization of glucose to meet increased metabolic rate. Moreover, they reported reduction in lipid level in the different tissues due to the accelerated hydrolysis of lipid in order to cope with the increased energy demand.

Exposure to heavy metals such as Cu and Cd can inhibit some physiological activities by decreasing the uptake of amino acids, which could decrease their distribution on tissues (Viarengo *et al.*, 1980). In the present study, the non-essential amino acids decreased significantly in treated *B. plicatilis* with $CdCl_2$ and $CuSO_4$ when compared to their control, except for aspartic acid, while the essential amino acids showed elevated level than control except for Arginine and Threonine. This could be due to the toxic effect of these heavy metals on the protein metabolism in

the living organisms (Wang *et al*., 2014). Lin and Li, (1991) found that the amount of essential amino acids was higher than non-essential amino acids in copepod *Calanus sinicus* under the effect of Cu and Cd which explains the effect of heavy metals on protein synthesis.

In the present study, the level of methionine increased significantly in treated *B. plicatilis* with $CdCl_2$ and $CuSO_4$. Wu *et al.* (2017) found that methionine supplementation improved growth performance. This finding illustrated the significant increase of body length and width of *D. labrax* larvae at 30 dph reared on treated *B. plicatilis*. In the present study, phenyl alanine and histidine increased in treated *B. plicatilis* with $CdCl_2$ and $CuSO_4$. Dean *et al.* (1997) reported that some amino acids may scavenge reactive oxygen species (ROS). According to the given results, it is suggested that amino acid composition be regarded as a sensitive biochemical indicator of Cu and Cd toxicity because of the effect of these metals on protein synthesis, so they may act as a signal of physiological stress in marine organisms subjected to heavy metal pollution.

Several studies demonstrated that fatty acid constituents can be altered by anthropogenic activities due to the aquatic environment pollution (Penha-Lopes *et al*., 2009; Cheung *et al*., 2010; Perrat *et al*., 2013). Recent studies provided that Cd decreased omega-3 contents (Merad *et al*., 2017). This study showed that the total fatty acid contents of *B. plicatilis* treated with CdCl2 (not with CuSO4) significantly decreased when compared to non-treated control which can explain why Cd is more toxic than Cu as a heavy metal on living organism, as it acts on reducing their nutritional value (Ługowska and Kubik, 2011, Drudi *et al*., 2017). In the present result, Eicosatrienoic acid (C 20:3ω3) and Eicosapentaenoic acid (C 20:5ω3) were not detected, while Arachidonic acid (C20:4 ω6) was significantly decreased in treated *B. plicatilis* with CdCl2. Filimonova et al. 2016, and Signa et al. 2015 have reported that the Eicosapentaenoic acid level is diminished with the increasing contamination content. Fokina *et.al* (2013) reported that EPA and ARA are associated with adaptation improvement. This may indicate the possibility to use fatty acids composition parameters as biomarkers reflecting the adverse effect of the metals.

CONCLUSION

In conclusion, *D. labrax* larvae fed on *B. plicatilis* and exposed to sublethal concentration of Cd and Cu heavy metals showed marked morphometric changes, increased in the rate of death and decreased some biochemical compositions.

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