

Studies on clove as a potential antidote for metal intoxication in *Tilapia mossambica*

Dhanya Pulikkottil Rajan^{1*} and A.B. Surya²

¹School of Industrial Fisheries, Cochin University of Science and Technology, Kochi - 682 016, Kerala, India

²Post graduate Department of Aquaculture, St. Albert's College, Kochi - 682 018, Kerala, India

*Corresponding author: Email: dhanyapulikkottil@yahoo.com

ABSTRACT

The present study was carried out to reduce the concentration of lead and cadmium in the liver of *Tilapia mossambica* using the chelating property of clove. Lead (Pb) and cadmium (Cd) levels were estimated using Inductively Coupled Plasma Spectrometer - ICAP 6300 Duo. Reduction in Pb content from 3.40 ± 0.05 ppm (initial value) to 0.8 ± 0.03 ppm and Cd content from 1.68 ± 0.02 ppm (initial value) to 0.1 ± 0.01 ppm was observed in the liver of fishes fed with clove incorporated feed. The results thus revealed a reduction in lead and cadmium content by 78.70% and 96.15%, respectively in the clove fed fishes. Hence, clove incorporated feed can be used to reduce the lead and cadmium content in fish (**Keywords:** Lead, cadmium, *T. mossambica*, clove).

INTRODUCTION

Heavy metals such as cadmium, zinc, mercury, chromium and copper cause pollution particularly in the ponds, lakes and river systems in zones where effluents released from industries, sewage and agricultural practices drains. Organisms accumulate metals from polluted environment. Among the animal species, fishes are the inhabitants that cannot escape the detrimental effects of these pollutants (Basha and Rani, 2003). Finfish and shellfish accumulate chemicals from the polluted environment. In recent years, high levels of cadmium have been observed in marine products (Lakshmanan, 1988). Important source of cadmium in the water bodies are waste discharge from zinc smelting, electroplating industry, pigment plants and effluents from battery and alloy production units. The normal concentration of cadmium in seawater is 0.02 mg L^{-1} and the concentration toxic to the aquatic life is in the range of 0.01 ppm to 0.1 ppm (Iyer *et al.*, 2002). Their abnormal levels in seafood pose hazards to public health due to its consumption. However, the level of cadmium in cuttlefish and squid often exceeded the limit and Italy and Spain rejected some consignments from India in the 80's (Iyer *et al.*, 2002). Cadmium is mainly concentrated in the liver and gut of cephalopods (Iyer *et al.*, 2002). Similarly, lead is pervasive and widely distributed

pollutant with no beneficial biological roles (Swarup *et al.*, 2005). Lead (Pb) affects mainly the nervous system especially the central nervous system (CNS) (Dorea and Donangelo, 2006). In Siddha system, herbal antidotes play a significant role as they neutralize the toxic effect of the poisonous substance and thus serve as an elixir of life (Rajalakshmi, 2012). Clove oil possesses free radical scavenging and iron chelating properties and inhibits lipid peroxidation to a greater extent (Chaieb *et al.*, 2007; Jirovetz *et al.*, 2006; Yadav and Bhatnagar, 2007). Hence, in the present study, the chelating property of clove was used to reduce the lead and cadmium concentration in *Tilapia mossambica* collected from Pizhala island region of Ernakulam, Kerala.

MATERIAL AND METHODS

Study area

Pizhala island situated on the northern side of Kochi and the southern side of Varapuzha in Ernakulam, Kerala, has been made of sedimentary sand accumulated by River Periyar at the time of flood. The Eloor and Edayar industrial areas, a home for more than 140 industrial units, discharge the effluents in to river Periyar. The effluent laden river Periyar affect the biota in and around Pizhala.

Fish

Tilapia mossambica, commonly known as Tilapia was selected for the present study due to its availability throughout the year and suitability for toxicity test. The Teleost fish belonged to the family Cichilidae, order Perciformes and subclass Actinoptergii. Live fish (30 Nos.) with an average length of 8 ± 1 cm and average weight of 20 ± 1 g were collected from Pizhala region using cast net and transported to the wet lab in a well oxygenated polythene bag containing clean pond water.

Feed preparation

Fish meal ingredients consisted of groundnut oil cake, shrimp meal, wheat flour, tapioca powder, vitamin and mineral mixture. The ingredients were well mixed with potable water, steamed and allowed to cool. Fish oil and vitamin E tablets were added and mixed well. The dough was then passed through the pelletizer, dried and stored in an airtight container and used as the control feed. Feed containing clove was prepared by the addition of 7g of clove to the normal feed mixture, mixed well, passed through the pelletizer, dried and stored in an airtight container.

Experimental set up

The fish were acclimatized in the tank water for 1 h and then used for the experiment. Aeration was provided in the tanks. The fishes in control tank were fed with normal feed and the fishes in the experimental tank were provided with clove containing feed. Water was exchanged daily to prevent accumulation of ammonia in the medium. The temperature and pH of water was maintained at $26 \pm 1^\circ\text{C}$ and 7.00 ± 0.50 , respectively. The experimental set up was maintained for a period of 3 months (90 days) and sampling was carried out at the end of every month. Liver samples were removed for the estimation of lead and cadmium.

Heavy metal analysis

The liver sample (0.25 g to 0.50 g) from the experimental and control fish were treated with nitric acid (8 mL) and H_2O_2 (2 mL), kept for 10 min and digested in microwave digestion chamber. After

digestion the sample was then made up to 100 mL. Digested sample was analyzed in ICP (Inductively Coupled Plasma Spectrometer – ICAP 6300 Duo).

Statistical analysis

SPSS 17.0 software program was used for the analysis of data. The results were analyzed utilizing the software for the unpaired *t* test. Comparison was made between each experimental group and the control group (without clove).

RESULTS AND DISCUSSION

Temperature of the water was maintained at $26 \pm 1^\circ\text{C}$ throughout the study period of 3 months. The pH was monitored daily and the average pH was 7.00 ± 0.50 . The initial level of lead and cadmium in the liver of Tilapia were analyzed before starting the experiment. The change in the metal level was monitored at the end of every month for a period of three months (Table 1). The fish caught from Pizhala region had an initial lead content of 3.40 ± 0.05 ppm. The results revealed a reduction in lead concentration in both clove treated and control (untreated) samples from initial concentration (Table 1). Further, a decline in Pb content was observed in the liver of the experimental fish (clove fed) when compared to the Pb concentration in the control fish (feed without clove) (Tables 1 and 2). A significant difference ($p < 0.05$) was observed in lead levels in the liver of tilapia with and without administration of clove enriched feed. The percentage reduction in lead content was more pronounced in clove treated fish (Table 2).

The initial level of cadmium in the liver of Tilapia was 1.68 ± 0.02 ppm (Table 1). However, the FSAI (2009) recommended permissible limits for cadmium in muscle meat of fish was much less (0.05 ppm). In a similar study by Mehoul *et al.* (2019), the mean concentrations of Cd and Pb in Sardine were found to be 0.55 and 2.13 mg kg^{-1} wet weight, respectively, while in swordfish, the concentrations were 0.57 and 3.9 mg kg^{-1} wet weight, respectively. A significant difference ($p < 0.05$) was observed in cadmium levels in the liver of Tilapia with and without administration of clove enriched feed. The decrease in cadmium content in

clove fed fish was higher (96.15%) as compared to the control fish (88.86%). Clove treatment was more effective in reducing the Cd load (96.15%) than Pb (78.70%) in the fish samples.

Table 1. Lead and cadmium concentration in the liver of *Tilapia mossambica*

Sampling period (Months)	Lead content (ppm)		Cadmium content (ppm)	
	DN	DC	DN	DC
0 (Before treatment)	3.40 ± 0.05 ^a	3.40 ± 0.05 ^a	1.68 ± 0.02 ^a	1.68 ± 0.02 ^a
1	3.20 ± 0.03 ^a	3.10 ± 0.01 ^a	1.30 ± 0.06 ^a	1.00 ± 0.05 ^a
2	2.30 ± 0.11 ^a	1.20 ± 0.07 ^b	1.00 ± 0.19 ^a	0.60 ± 0.02 ^b
3	0.90 ± 0.05 ^b	0.80 ± 0.03 ^c	0.20 ± 0.04 ^b	0.10 ± 0.01 ^c

Values expressed as mean ± SD; Values with different superscripts in the same column indicate significant difference at $p < 0.05$; DN - depuration with normal feed; DC - Depuration with clove enriched feed

Table 2. Percentage reduction in lead and cadmium in the liver of *Tilapia mossambica*

Sampling period (Months)	Percent reduction in lead (%)		Percent reduction in cadmium (%)	
	DN	DC	DN	DC
1	6.806	10.93	27.29	40.45
2	31.67	64.56	39.13	62.25
3	75.40	78.70	88.86	96.15

DN - depuration with normal feed; DC - Depuration with clove enriched feed

CONCLUSION

Overall results of the present preliminary study revealed that the administration of clove incorporated feed reduced the Cd and Pb loads in the liver of *Tilapia mossambica*. Further intensive studies in the same area may help in the formulation and application of clove enriched fish feed in the reduction of heavy metal load in fishes.

REFERENCES

Basha, P.S. and Rani, A.U. 2003. Cadmium-induced antioxidant defense mechanism in freshwater teleosts

Oreochromis mossambicus (Tilapia). *Ecotox. Environ. Safe.* **56**, 218 - 221.

Chaieb, K., Hajlaoui, H. and Zmantar, T. 2007. The chemical composition and biological activity of clove essential oil, *Eugenia caryophyllata* (*Syzygium aromaticum* L. Myrtaceae): A short review. *Phytother. Res.* **21**, 501 - 506.

Dorea, J.G. and Donangelo, C.M. 2006. Early (in uterus and infants) exposure to mercury and lead. *Clin. Nutr.* **25**, 369 - 376.

FSAI (Food Safety Authority of Ireland). 2009. Mercury, lead, cadmium, tin and arsenic in food. Toxicology fact sheet series, Issue no.1. (<https://www.fsai.ie/WorkArea/DownloadAsset.aspx?id=8412>)

Iyer, G., Kandoran, M.K., Thomas, M. and Mathew, P.T. 2002. Quality assurance in seafood processing. Central Institute of Fishery Technology and Society of Fisheries Technologists, Cochin, India.

Jirovetz, L., Buchbauer, G., Stoilova, I., Stoyanova, A., Krastanov, A. and Schmidt, E. 2006. Chemical composition and antioxidant properties of clove leaf essential oil. *J. Agric. Food Chem.* **54**, 6303 - 6307.

Lakshmanan, P.T. 1988. Cadmium in seafood products. *Fish. Technol.* **25**, 142 - 146.

Mehouel, F., Bouayad, L., Hammoudi, A.H., Ayadi, O. and Regad, F. 2019. Evaluation of the heavy metals (mercury, lead, and cadmium) contamination of sardine (*Sardina pilchardus*) and swordfish (*Xiphias gladius*) fished in three Algerian coasts. *Vet. World* **12**, 7 - 11.

Rajalakshmi, P. 2012. Antidotes for poisonous food substances. *J. Pharm. Res.* **5**, 1432 - 1434.

Swarup, D., Patra, R.C., Naresh, R., Kumar, P. and Shekhar, P. 2005. Blood lead levels in lactating cows reared around polluted localities; transfer of lead into milk. *Sci. Total Environ.* **349**, 67 - 71.

Yadav, A.S. and Bhatnagar, D. 2007. Free radical scavenging activity, metal chelation and antioxidant power of some of the Indian spices. *Biofactors* **31**, 219 - 227.

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