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Short Communication

A comparative study on manganese accumulation in liver, kidney, fatty tissues including muscle of rainbow trout (*Oncorhynchus mykiss*) fed with different levels of manganese in the diet

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Most minerals, occurring in aqueous environments, play a key role in the survival rate of aquatic living organisms. However, life situation of the living organisms would be under attack when the contents of these elements exceed than the specified limit because of various reasons (Amini Ranjbar, 1994). Bioaccumulation of the elements in living organisms and their excess concentration than standard limit would result in acute and chronic biological impacts in their tissues (Kimmo *et al*., 2004). Metals like manganese, copper, zinc, and iron are essential elements in metabolic activities of living organisms at ppm levels and maintaining body metabolism as well. Naturally, the elements are considered as enzyme formers at very poor levels to increase fish health status. They should be taken through water, food and sediments; however, they would lead to intoxication when taken at higher levels (Chen *et al*., 2008). It has been well documented that fishes are considered as good indicators for long term monitoring of heavy metal bioaccumulation in marine environment due to their access to different dietary levels (Has-Schon *et al*., 2008). Mineral elements are important from different metabolic aspects. Beyond their effects on bones strengthening, these elements have also beneficial effects on homeostasis and maintenance of the fish body with their aqueous surrounding environment through nervous system and endocrine glands. In addition, these elements act as formers of most enzymes and pigments and in some metabolic activities and energy transportation (Heen *et al*., 1993). Tissues of aquatic organisms are very low in manganese level; however, most tissues have very little manganese accumulation. Bones, liver, pancreas and pituitary gland showed to have the highest manganese concentrations than other organs (Salek Yosefi, 2000). As with the magnesium, manganese is also considered as enzyme activators, as several phosphyt transferases and dicarboxylysis are activated by this element. Usually, aquatic organisms can uptake manganese from the water and provide the required level of manganese from dietary foods (Salek Yosefi, 2000). Normally, this element has less toxic effect to fish body than iron. Clinical symptoms of manganese are hyper-excitation, imbalanced swimming, discoloration of fins and sensory barbels. Other symptoms which is seldom observed includes fin distortion, cataract and the presence of adhesive filaments on fish skin. At final stage, the fish will paralyses and after a few hours, it will convict to the death (Van Duijn, 2000). This element is necessary for the regulation of fish nervous system, bone development and reproduction. Also, manganese has an efficient role in carbohydrate metabolism and is needed for fish health enhancement (Malekniya and Shahbazi, 1996). Manganese, a nutritional element, is essential for the survival of all kinds of animal species but at a low level. Manganese loss would result in a remarkable decline in growth rate, abnormal development of the fish tail, shortening of the body and disturbances in reproduction system of rainbow trout. On the whole, the loss of manganese in fish body would lead to malformation, abnormality of the bones and retardation in the synthesis of blood cells. In a previous study by Ogino and Yang (1980), it has been observed that rainbow trout fed with diets containing 4 mg kg^{-1} of manganese had less growth rate than those fed with diet containing 13 mg kg^{-1} of manganese. Recent investigations indicate that a few studies have been focused on mineral bioaccumulation, for example manganese, in rainbow trout in Iran. This survey aimed to study the accumulation of manganese at different levels in liver, kidney, fatty tissues including muscle of rainbow trout.

The experiment was performed in fish farming center of Arab Kheil at the countryside of Amol city for 90 days from July to October 2010. Four diets were prepared for 204 rainbow trout samples. Experimental feeding diets included: the control treatment which contained 29 mg kg^{-1} of manganese. This level of manganese occurs naturally in the principle composition of the feeding diets. Treatments 1, 2 and 3 contained 48, 78, and 98 mg of manganese sulphate per kg of food, respectively (Halver, 1988). Feeding to the fish was based on 3% of the total body weight and conducted three times a day (at 8 am, 12 am, and 6 pm) according to the water temperature. The amount of manganese sulphate in each experimental feeding diet was measured in accordance to the manganese content in the control treatment. Then each experimental dose of manganese sulphate was mixed well with 1 kg of factory prepared food in order to spread the manganese homogenously in a given diet. After two weeks acclimation, fish samples in each treatment with average weight of 50 g and density of 51 individuals in each farming channel (100×200×1500 cm^3) were fed with different feeding diets for three months. At the end of the farming period, manganese bioaccumulation in liver, kidney, fatty tissues including muscle of fish samples in treatments and the control were compared. Twelve fish from each treatment (4 from each replicate) were randomly selected and then placed into dark polyethylene bags. Afterward, samples were frozen at -20ºC and transported to the Pasargad Research Laboratory in Tehran. After transportation, preparation and analysis processes on fish samples were conducted. To do so, muscle, liver, kidney and fatty tissues of the fish were separated according to the implementation guideline. For heavy metal measurement, samples were derived into ash (Tacon, 1990):

$$\mathrm{Ash}(\%) = [\frac{(\mathrm{A}+\mathrm{W_b})-\mathrm{W_b}}{\mathrm{W_s}}] \times 100$$

Where A is the amount of ash; W_b: the weight of tiled bowl; W_s: the weight of sample.

Hydrolysis method was applied to extract the fat from the muscle tissue (Wiehlloughby, 1990):

$$\mathrm{Fat}\ (\%) = [\frac{\mathrm{W_p} - \mathrm{W_f}}{\mathrm{W_s}}] \times 100$$

Where $W_p$is the weight of empty petri dish; $\mathrm{W_f}$: the weight of petri dish containing fat; $\mathrm{W_s}$: the weight of sample. To determine manganese content in kidney, liver, fatty tissues including muscle of the fish, an atomic Philips absorption apparatus (Model PU, 9400) was used. Data were subjected to the One-Way ANOVA using statistical software package of SPSS. Mean comparisons were performed by Duncan's multiple range test. Data calculation and drawing of diagrams were done by software package of Excel. Temperature averaged between 16 and 17ºC and it showed no remarkable changes during farming period. pH value ranged around 7.5 and water flow rate was 4 l^{-1} $second^{-1}$. Water oxygen content ranged from 8.7 to 9.9 mg l^{-1}. According to the obtained results, fish samples treated by the highest level of manganese in dietary food (treatment 3) revealed the highest manganese accumulation in liver, kidney, fatty tissues including muscle, whereas the control treatment detected the lowest content of manganese. Results evidenced the increased accumulation of manganese in tissues of 98mg-treated fish during a 90-day period. Results of One-Way ANOVA indicated significant differences (p<0.05) between treatments and the control.

Results of comparisons of manganese content in liver, kidney, fatty tissues including muscle are depicted in Fig. 1, 2, 3 and 4. Fish samples in treatment 3 had the highest mean level of manganese accumulation in their tissues as compared to the control fish. Moreover, comparison of manganese content in the studied tissues revealed significant differences (p<0.05) among various treatments and the control.

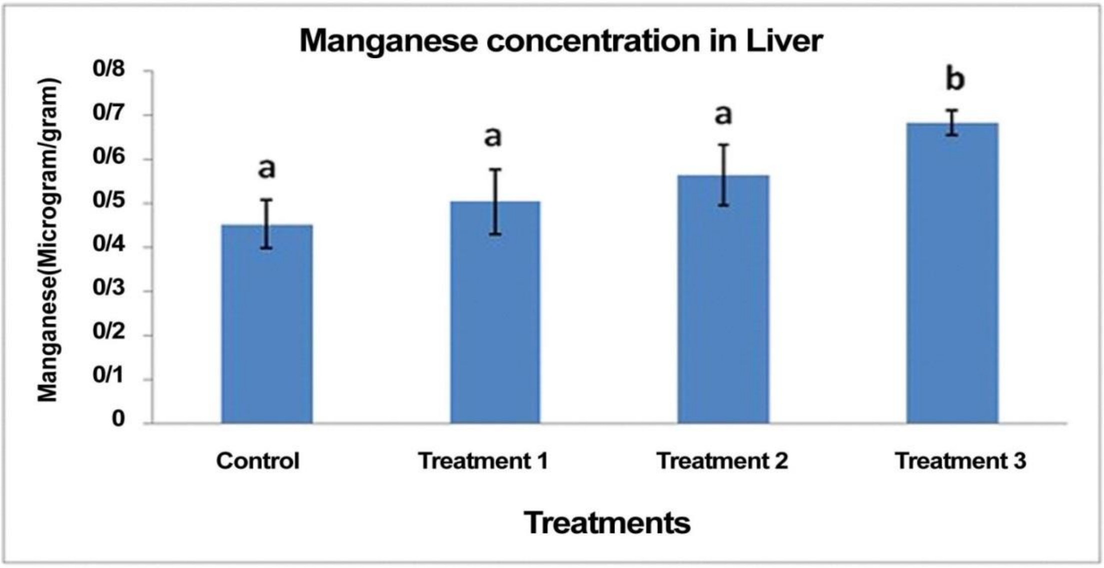

Figure 1: Comparison of manganese concentration (mean±SD) in liver of rainbow trout (Different characters show significant difference at *p*<0.05 using Duncan's multiple range test).

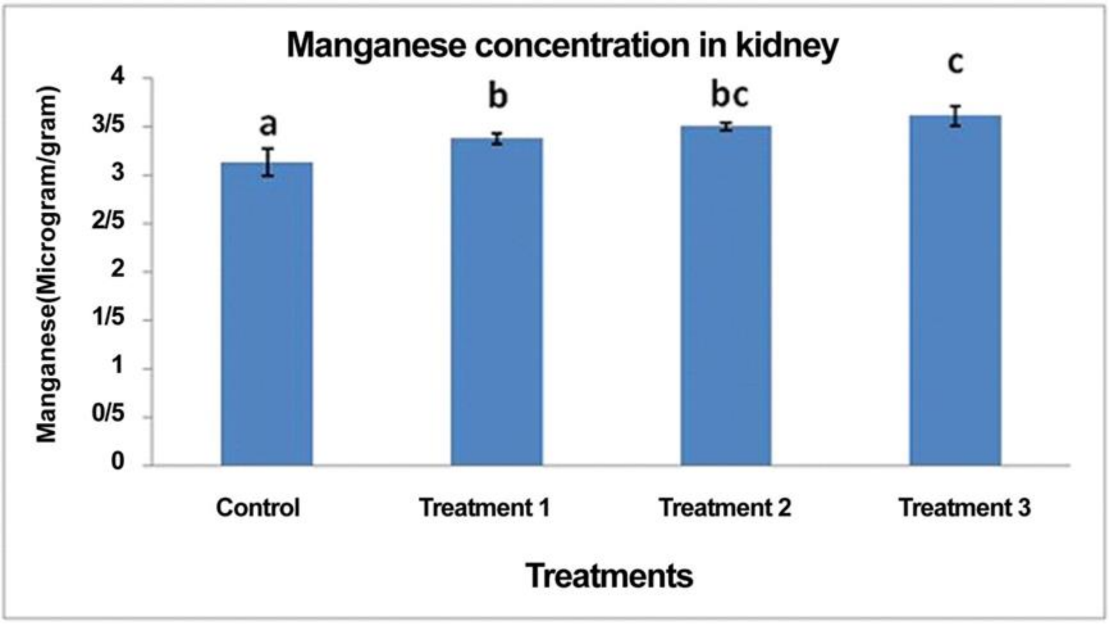

Figure 2: Comparison of manganese concentration (mean±SD) in kidney tissue of rainbow trout (Different characters show significant difference at *p*<0.05 using Duncan's multiple range test).

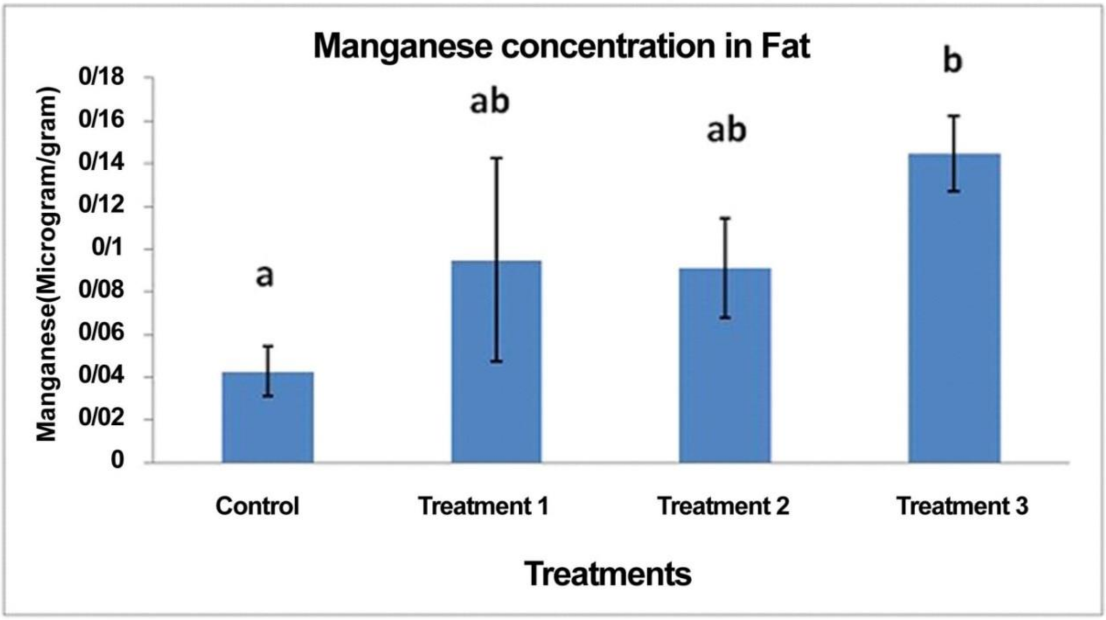

Figure 3: Comparison of manganese concentration (mean±SD) in fatty tissue of rainbow trout (Different characters show significant difference at *p*<0.05 using Duncan's multiple range test).

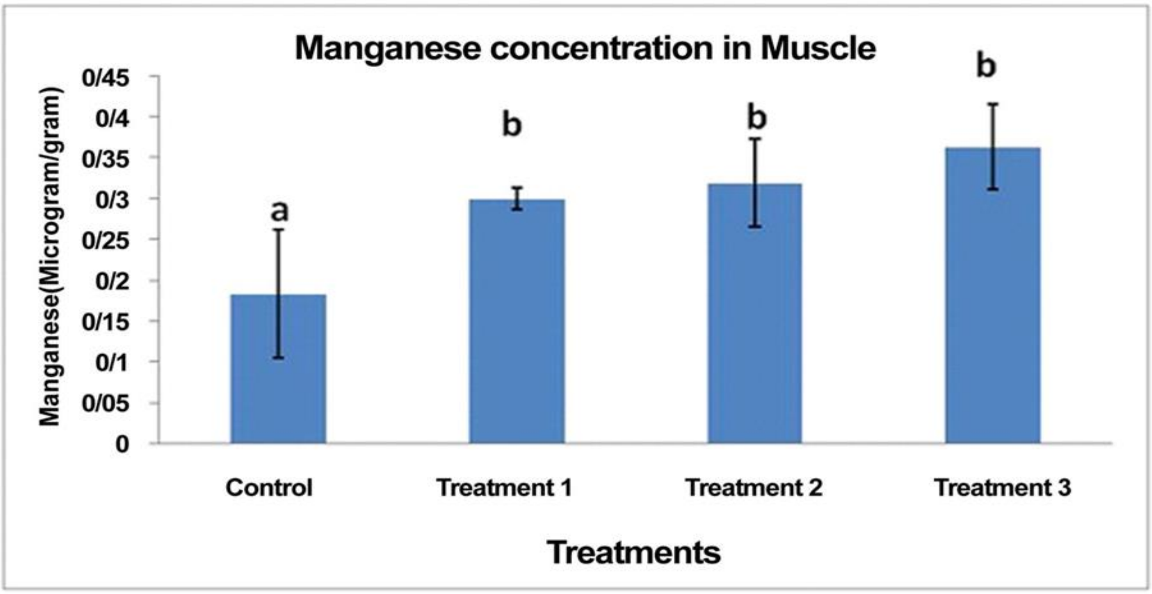

Figure 4: Comparison of manganese concentration (mean±SD) in muscle tissue of rainbow trout (Different characters show significant difference at *p*<0.05 using Duncan's multiple range test).

Regarding to the obtained results, manganese accumulation in body tissues increased parallel with increase in its concentration in dietary foods. Results of statistical analysis using One-Way ANOVA revealed significantly ($p<0.05$) lower content of manganese in control fish samples as compared to other treatments. Fish samples in treatment 3 indicated the highest level of manganese in their body tissues. Also, mean comparisons of manganese content showed significant ($p<0.05$) differences among various treatments.

In this study liver, kidney, fatty tissues including muscle of the rainbow trout were examined. Based on the results obtained in the present study, addition of manganese to the fish dietary food increased the accumulation of this element in their body tissues in comparison to the control treatment. As fish muscle tissue is of importance for human consumption, the tissue was studied here. Other fish tissues were also examined in order to investigate any absorptive differences with muscle tissue. There was a significant relationship between manganese concentration in body tissues and feeding diets. Previous studies demonstrated that mean concentration of manganese is different in various tissues. Results of the present work presented that fish samples in treatment 3, which had received the highest level of manganese in their feeding diet, had the most content of manganese in their studied tissues than other treatments and the control. Furthermore, manganese level was less than the allowable limit admitted by the World Health Organization, implicating no problem in the quality of fish meat (Sing and Ferns, 1978). Approximately, 5-10 mg day^{-1} of manganese is recommended as the safe range for the human health. Manganese might cause fish intoxication by the absorption from the feeding diets. As expressed by previous researchers, drinking water contains 100 µg l^{-1} of manganese is recommended for human health and there would be inevitable issues such as psychosis, mental and nervous disturbances as well as a considerable decline in the absorption of other minerals like calcium, copper, iron, magnesium and zinc.

Manganese absorption in human body depends on the age, feeding diets, iron content and other minerals. Around 3 to 8 % of manganese is taken in by means of alimentary duct. Totally, daily manganese uptake by means of feeding diets, drinking water and respiration is estimated to be 3-8 µg, 0.1 to 24, and 400 ng, respectively. It has been reported that manganese is absorbable to human body by respiration system (Heen *et al*., 1993). Manganese might cause fish intoxication by the absorption from the feeding diets. As expressed by previous researchers, drinking water contains 100 µg l-1 of manganese. Lethal concentration of manganese in rainbow trout fish is 0.1 g l^{-1} and this metal exhibited its toxicity effects in fish body when adding as manganese sulphate (0.5 g l^{-1}) and manganese chloride (0.33 g l^{-1}) (Heen *et al*., 1993). Having been entered to the blood circulation, manganese influxes to the brain before it is metabolized in the liver. Therefore, this element causes undesirable effects on central nervous system. As manganese can be excreted by means of bilious system, the element poissesses more simple mechanism in feeding system (Esmaeli Sari, 2002).

Generally, differences in metal accumulation of various body tissues might be due to the differences in their ecological demands and metabolic activities. According to the presented results, more bioaccumulation of manganese in body tissues of those fish treated by 98 mg kg^{-1} might be attributed to the high content of manganese in their feeding diets. In a previous research by Yamamoto *et al*., (1983), manganese bioaccumulation showed a direct relationship with the weight of rain bow trout fish samples. Based on the results obtained here, the most level of manganese accumulated in kidney tissues of the rainbow trout than other tissues. Like other minerals, manganese can accumulate in some fish tissues. Many previous researchers demonstrated that manganese bioaccumulation in fish muscle is less than the accumulations of minerals like iron, copper, zinc, and magnesium (Sing and Ferns, 1978).

This element can aggregate in body tissues including heart, kidney, gills, muscle, intestine, and liver (Stranah and Andreji, 1998). Freshwater fish can retain ions in their kidney and excrete excess water from their bodies in order to survive. In addition, organs like heart and kidney are the locations in which trace elements can accommodate in most fish species (Olsson, 1998).

In the surveys on heavy metal concentrations of zinc, iron, copper, manganese, nickel and cadmium (dry weight) in west white leg shrimp caught from northwest coast in Mexico, it has been declared that most heavy metals can accumulate in hard tissues and muscle. Several investigators believe that heavy metals can accumulate in various body tissues of the living organisms but the animal tissues can tolerate against the excess bioaccumulation of these elements as much as possible if metal content exceeds the threshold level (Rogers and Richards, 2003).

According to the obtained results, it can be concluded that there is a significant relationship between manganese concentrations in liver, kidney, fatty tissues including muscle of rainbow trout. Manganese mean content is less than the standard limit of FAO which contradicts any interdict in the consumption of rainbow trout. Significant differences were observed among manganese contents in treated fish samples and the control. Manganese content in kidney tissues showed the highest level than other tissues. Until now, several studies have been focused on the accumulation of manganese in rainbow trout fish throughout the world. In addition, no research has been conducted on manganese bioaccumulation in body tissues of rainbow trout fish so far in Iran.

References

Amini Ranjbar, G., 1994. A survey on heavy metal accumulation (Zn, Cu, Pb, and Cd) in surficial sediment of Anzali Wetland. *Iranian Journal of Fisheries Sciences*, 3, 5-26. (in Persian).

Chen, Z.N., Zhang, L., Zhuo. and Tang, B., 2008. Catalytic kinetic methods for photometric or fluorometric determination of heavy metal ions. *Journal of Microchim Acta*, 164(**3-4**), 311- 336.

Esmaeli Sari, A., 2002. Pollutants, hygiene and standards in natural environment. Naghsh Mehr Publication. Tehran. pp. 149-156.

Halver, J.E., 1988. Fish nutrition. academic press. United State of Amireca. 494P.

Has-Schon, E., Bogut, I., Rajkovic, V., Bogut, S., Cacvic, M. and Horvatic, J., 2008. Heavy metal distribution in tissues of six fish species included in human diet, inhabiting freshwaters of the nature park (Bosnia and Herzegovina). *Journal of Arch Environmental Contamination Toxicology*, 54, 75-83.

Heen, K., Monahan, R.L. and Utter, F., 1993. Salmon aquaculture. Fishing News Books. 278P.

Kimmo, A.M., Pentinen, O.P. and Jussi, V.K., 2004. Pentachlorophenol bioaccumulation and effect on heat production on salmon eggs at different stages of development. *Aquatic Toxicology*, 68,75-85.

Malekniya, N. and Shahbazi, P., 1996. General biochemistry. Tehran University Publication. Tehran, pp. 87-104.

Ogino, C. and Yang, G.Y., 1980. Requirement of rainbow trout for dietary zinc. *Bulletin of the Japanese Society of Scientific Fisheries*, 44, 1015-1018.

Olsson, P.E., 1998. Disorders associated with heavy metal pollution in fish disease and disorders. Vol. 12. Noninfectious disorders. Neatherland J.F.WOO.P.T.K.(Eds). CAB International Publishing, Oxford, England. 386P.

Rogers, J.T. and Richards, J.G., 2003. Ion regulatory distruption as the acute toxic mechanism for rainbow trout. *Aquatic Toxicology*, 64, 215–234.

Salek Yousefi, M., 2000. Feeding of farming aquatics (coldwater, warm water and shrimp), Cultural Institute of Alani Publications, Tehran, 318P.

Sing, S.M. and Ferns, P.N., 1978. Accumulation of heavy metals in rainbow trout *Salmo gairdneri* on a diet containing activated sewage sludge. *Journal of Fish Biology*, 13, 277–86.

Stranah, I. and Andreji, J., 1998. Levels of cobalt, nickel, copper and manganese in fish the Danub River. Slovak. 66P.

Tacon, A.G.J., 1990. Standard methods for the nutrition and feeding of farmed fish and shrimp. Argent Laboratories Press. pp. 4-24.

Van Duijn, J.R.C., 2000. Diseases of fishes. Narendra Publishing House. Delhi, India. 174P.

Wiehlloughby, S., 1990. Salmonid farming. Fishing News Books. 329P.

Yamamoto, H., Satoh, S., Takeuchi, T. and Watanabe, T., 1983. Effect on rainbow trout of manganese or trace element from fish meal diet. *Bulletin of the Japanese Society of Scientific Fisheries*, 49, 287 – 293.