ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology 7 (1): 139-143, 2008
ISSN 1682-296X

Natural Bioremediation of Heavy Metals Through Nematode Parasite of Fish

[1]Rafia Azmat, [2]Shahina Fayyaz, [2]Nasira Kazi, [3]Syed Junaid Mahmood and [4]Fahim Uddin
[1]Jinnah University for Women, Karachi, Pakistan
[2]National Nematological Research Centre,
[3]PCSIR Laboratories Karachi, Pakistan
[4]Department of Chemistry, University of Karachi, Karachi, Pakistan

Abstract: Parallel analysis of heavy metals (Pb, Cd, Hg, As, Zn and Fe) in muscles and guts of fishes, seawater and fish parasites were detected by atomic absorption Spectrophotometry. The bioaccumulation potential of heavy toxic metals was assessed in the *Echinocephalus* sp. and *Ascaris* sp. which is, reported as natural bioremediator of heavy metals in *Liza vaigiensis* from Karachi coast. Investigation suggests that infected fish contain low concentration of heavy metals in their muscle as compared to non - infected one. The high level of toxic metals in *Echinocephalus* sp. and *Ascaris* sp. within its host suggests that these nematode parasites are sensitive indicator of heavy metals in aquatic ecosystem showing sharing of more burden of environmental pollution of sea and also act as bioremediator of heavy metals in fish.

Key words: Metals, nematode parasite, fish, bioremediator

INTRODUCTION

Pollutants might promote increased parasitism in aquatic animals, especially fish by impairing the host's immune response or favoring the survival and reproduction of the intermediate hosts (Khan and Thulin, 1991). Parasites are naturally occurring organism (Barus *et al*., 1999), attracting increasing interest as potential indicator of environmental quality due to variety of ways in which they respond to anthropogenic pollution (Barak and Mason, 1990; Sures, 2001). Deteriorating state of the environment in the aquatic resources is reflected by the increased degree of fish nematode parasite infection and elevated metallic pollutants levels. Some trace toxic metals have been found to accumulate to higher extent in parasite than in tissues of their host organism and to demonstrate a good suitability as a biomonitor of environmental pollution (Schludermann *et al*., 2003). The parasitic infection rate in fish is often concern as indication of the degree of immunity of organism, or community or system subjected to exogenous, potentially recurring stimulus (Szefer *et al*., 1998).

Most reports of pollution effects on endoparasites suggest increased parasitism in fish hosts. This also implies to fish living in the areas which receive industrial/thermal effluents. Parasite might in turn enhance their hosts, susceptibly to pollutants (Chibani *et al*., 2001).

Several types of pollutants, including domestic sewage, pesticides, polychlorinated biphenyls, heavy metals, pulp and paper effluents, petroleum aromatic hydrocarbons, acid rain and others are known to effect marine life (Ucman *et al*., 2001). Many of the later are parasitized and under natural environmental condition most fish parasites are believed to cause little or no harm. However, chronic exposure to pollutants over a period of time cause physiological, behavioral and biochemical host changes that ultimately can influence the prevalence and intensity of parasitism (Khan and Thulin, 1991). Brotheridge *et al*. (1998) reported that Trout were collected from two different sampling stations, one site adjacent to the river Otra South of Evje where Nickel mined and trout likely to be infected with parasites nematode, *Eustrogylidies* sp. due to the presence of Ni and Cu in fish and other from down stream was non infected.

The objective of this study to asses the degree of infection in marine fish and to determine the concentration of heavy metals in water, parasites and tissues of fish.

MATERIALS AND METHODS

Sixty-seven specimens of *Liza vaigiensis*, were targeted to study the concentration of heavy metals and parasitic infection. Fishes were collected from fish harbor Karachi in July 2006 in early morning through trawler with water samples. Samples were collected in sterile flasks and keep air tight to avoid any contamination in laboratory. Each fish specimen was washed with deionized water and then surface dried with filter paper.

The examination was aimed to study the infection in fishes by nematode parasites and heavy metal found in

Corresponding Author: Rafia Azmat, Jinnah University for Women, Karachi, Pakistan

edible fish. Total Length (TL), Total Weight (TW) of fishes and infection intensity by the parasites were measured in individual fish sample. The total length of fishes were ranged from 7-10 inches where as weight were ranged from 100-150 g.

Investigation of nematode parasite: All fishes were dissected by ordinary method and gastrointestinal tract were removed, placed in a saline solution. Guts were dissected longitudinally and examined for nematode parasites under the binocular microscope. Nematodes collected from the gut were fixed in 70% alcohol, preserved in glycerin and 70% alcoholic mixture and cleared in lactophenol. Temporary mounts of nematodes were made in pure glycerin and examined under stereomicroscope. Photomicrographs were made with an automatic camera attached to a compound microscope using Nomarski's interference contrast system.

Detection of toxic metals: Water samples were digested in specific volume of nitric acid (conc.) and then sequentially evaporated, after filtration each sample were made up to 100 cm^3 with deionized water (conductivity below 1 $\mu S\ cm^{-1}$). Detection of heavy metals were made by Hitachi (H-28750-10) Atomic Absorption Spectrophotometer having band width of 0.2 nm and adjustable wavelength range 190-900 nm with 250 mm Elbert mount diffraction grating monochromator, burner was titanium head for air acetylene.

Nematode parasite of fish, tissue samples of different organs of fishes (wet sample) were digested using HNO_3 and H_2SO_4 for detection of heavy toxic metals like Pb, Hg, Cd, As, Zn and Fe in a closed system by atomic absorption Spectrophotometry.

RESULTS AND DISCUSSION

Heavy metals analysis of seawater, fish and fish nematode parasites showed that Pb, As, Cd, Hg, Zn and Fe were detected. Table 1 showed the concentration of heavy metals in seawater collected from different coastal beaches of Karachi Coast. Table 2 showed the bioaccumulation potential of heavy metals in muscles and guts of *Liza vaigiensis*. Table 2 also indicate that infected fish contain low concentration of heavy metal as compared to non-infected fish whereas Table 3 showed the heavy metals burden in parasite, *Echinocephalus* sp. and *Ascaris* sp.

Fishes were dissected for studying infection intensity by parasite. It was found that out of sixty-seven specimens twenty-four were infected by nematode parasite. Numerous nematode parasites were collected from the intestine of fish, which were identified as *Echinocepalus* Molin (1858) and *Ascaris* Linnaeus (1758). *Echinocephalus* sp. and *Ascaris* sp. are common parasites of fish of Karachi Coast for its pathogenic effects and reported here for the first time as a natural bioremediator of heavy metals within fish which shows more toxin burden in their body and help in the survival of the host in polluted environment.

In environmental impact studies certain organisms provide valuable information about chemical state of their environment not through their presence or absence but through ability to concentrate environmental toxins within their tissues (Tenora *et al*., 2000; Ucman *et al*., 2001; Sures *et al*., 2001). Table 1 showed that heavy metals are more prevalent and in higher concentration in the different beaches of Karachi Coast which may be attributed to the continuous discharge of industrial/domestic effluent in the aquatic resources (Trucekova *et al*., 2002; Tariq *et al*., 1993). Having high density, metals have more tendencies to accumulate in the tissue organs of animals as compared to essential micro and macronutrient ions as shown in Table 2 (Azmat *et al*., 2006a). According to EPA (Environmental Protection Agency) the residual tolerance level of Pb, Hg, Cd and As is 0.05 $mg\ kg^{-1}$, 0.1 $\mu g\ g^{-1}$, 0.05 $mg\ kg^{-1}$ and 0.04-0.15 $\mu g\ g^{-1}$, respectively which is comparable with the results of Table 1-2. This indicates the toxic effect of heavy metals for both human and fish, which was the provider of quality meat for human beings (Khansari *et al*., 2005).

Results in Table 3 showed that heavy metals concentration in non infected fish was higher as compared to infected fish by *Echinocephalus* sp. and *Ascaris* sp. It signify that parasites accumulate more concentration of toxic metals in the soft tissues of their body and provide natural remediation for pollutants to survive its host in contaminated environments. Heavy metals concentration differed significantly between organs of fish and nematode parasites *Echinocephalus* sp. and *Ascaris* sp.(Table 2, 3 and Fig. 1) with level to several fold higher in the parasite (Szefer *et al*., 1998).

Present results were supported by results of earlier investigators like Tenora *et al*. (1999) who reported more heavy metals burden like Pb, Cd, Cr and Ni in the parasites-host system in cyprinid fish. Similarly certain parasites, particularly intestinal *Acanthocephalus* of fish can accumulate heavy metals to concentration orders of magnitude higher than those in the host tissues (Sures *et al*., 1997). The amount of heavy metals was generally higher in parasites and lower in liver of perch infected with parasites than in the non-infected fish (Trucekova *et al*., 2002; Chibani *et al*., 2001).

Fig. 1: *Echinocephalus* sp. (A): Anterior region, (B): Posterior region *Ascaris* sp., (C): Anterior region, (D): Posterior region

Table 1: Concentration of heavy metals (μg g⁻¹) found in sea water from different sampling stations

Sampling station	As	Pb	Cd	Hg	Fe	Zn
Paradize point	125±12	30±3	11±2	04±3	80±12	25±03
Turtle beach	120±11	25±5	15±2	05±2	75±11	30±03
Sandspit	125±1	30±4	14±2	05±1	70±11	26±04
Clifton	128±13	36±5	17±2	09±1	73±11	32±03

Table 2: Heavy metal concentration (μg g⁻¹) in infected and non infected *Liza vaigiensis* fish

Name of species	Pb	As	Cd	Hg	Zn	Fe
Muscle						
Infected	0.28±0.01	0.18±0.01	0.15±0.01	0.34±0.01	0.20±0.01	0.20±0.01
Non infected	0.31±0.01	0.31±0.01	0.20±0.01	0.39±0.01	0.24±0.01	0.23±0.01
Guts						
Infected	0.23±0.11	0.24±0.01	0.09±0.02	0.21±0.01	0.11±0.01	0.23±0.01
Non infected	0.28±0.01	0.35±0.02	0.12±0.01	0.24±0.01	0.33±0.01	0.32±0.01

Table 3: Bioaccumulation of heavy metal potential (μg g⁻¹) in nematode parasite of fish

Nematode	Pb	As	Cd	Hg	Fe	Zn
Echinocephalus sp.	21±01	19±03	12±01	7±03	72±06	30±12
Ascaris sp.	25±03	22±02	15±02	9±01	80±07	32±11

Continuous discharge of industrial toxic material in water reservoir and presence of nematodes and heavy metals in fish can alter the metabolic and physiological function of the fish (Voegborlo *et al*., 1999). This also affects the quality of meat of fish. Because parasites (Barus *et al*., 2001) by definition require digestion of host tissue for their own nutrition and they rely on protrinases and oxidative damage for the tissue penetration and to supply their nutrients (Navratil *et al*., 1999*)*. Table 3 shows trace elements like Pb, Cd, As, Hg, Fe and Zn that have been found to accumulate to higher extent in nematode parasites as compared to tissues of host (Table 2) and demonstrate its suitability as a bioindicators of environmental pollution and suggest that they are sharing more burden in their soft tissues as well as persistent in contaminated environment (Azmat *et al*., 2006b) therefore act as bioremediator for fish (removing heavy metal)and help in the survival of fish with toxins. The parasitic infection rate in fish is often considered as an indicator of the degree of immunity of organism (Schludermann *et al*., 2003; Rolbiecki *et al*., 2000), Very high percentage of Fe in parasite is reported in Table 3 which may be attributed with lowering of iron (Table 2) in fish which in turns produced growth retardation, stunting and poor cognitive development in human and in animal.

CONCLUSION

Investigation revealed that parasites reducing environmental stress in fishes through bioremediation by concentrating the metals in their soft body tissues and minimizing the site disturbance within fish body compared with conventional clean up technologies.

ACKNOWLEDGMENT

Authors are thankful to Dr. Rafia Rehana Ghazi, Ex-Director, VPCI, PARC, University of Karachi, for the identification of fish nematodes and also for her helpful comments and suggestions.

REFERENCES

Azmat, R., S.S. Rizvi, R. Talat and F. Uddin, 2006a. Macronutrient found in some edible herbivorous and carnivorous fishes of Arabian Sea. J. Biol. Sci., 6: 301-304.

Azmat, R., Y. Akhter, R. Talat and F. Uddin, 2006b. Persistent of nematode parasites in presence heavy of metals found in edible herbivorous fishes of Arabian Sea. J. Biol. Sci., 6: 282-285.

Barak, N. and C.F. Mason, 1990. Mercury and lead in eels and roach: The effects of size, season and locality on metal concentration in flesh and liver. Sci. Total Environ., 92: 249-256.

Barus, V., F. Tenora, F. Kracmar, S. Proke and J. Dvoracek, 1999. Microelement content in males and females of *Anguillicola crassus* (Nematoda: Dracunculoidea). Helminthologia, 36: 283-285.

Barus, V., F. Tenora, M. Proke and M. Pe Az, 2001. Heavy metals in parasite host systems: Tapeworm vs. fish in Czech. In: *Ochrana zdravi*ryb (Health protection of fish). Vodaany, pp: 20-28.

Brotheridge, R.M., K.E. Newton and S.W. Evans, 1998. Presence of a parasite nematode (*Eustrongylidies* sp.) in brown trout from a heavy metal contaminated aquatic ecosystem. Chemosphere, 37: 2941-2943.

Chibani, M., M. Ziólkowska, A. Kijewska and J. Rokicki, 2001. *Pomphorrhynchus laevis* parasite of flounder *Platichthys flesus* as a biological indicator of pollution in the Baltic Sea. J. Mar. Biol. Assessment. UK., 81: 165-166.

Khan, R.A. and J. Thulin, 1991. Influence of pollution on parasite of aquatic animals. Adv. Parasitol., 30: 201-238.

Khansari, F.E., M.G. Khansari and M. Abdollahi, 2005. Heavy metals content of canned tuna fish. Food Chem., 93: 293-296.

Navratil, S., M. Palikova and R. Klement, 1999. *Anguilicolosis* of eels in water reservoirs of the Morava River basin. Helminthologia, 36: 129.

Rolbiecki, L., J. Rokicki and D. Wojtkiewicz, 2000. The first record of the nematode *Anguillicola crassus* in eel of the Gulf of Gadañsk (Poland). Oceanol. Studies, 29: 75-81.

Schludermann, C., R. Konecny, S. Laimgruber and J.W. Auteurs, 2003. Fish macroscopic parasites as indicator of heavy metal pollution in river sites in Austeria. Parasitology, 30: 201-238.

Sures, B., H. Taraschewski and J. Rokicki, 1997. Lead and cadmium contents of two cestodes *Monoboyhrium wageneri* and *Bothricephalus scorpii* and their fish host. Parasitol. Res., 83: 618-623.

Sures, B., 2001. The use of fish parasite as bioindicator of heavy metals in aquatic ecosystem: A review. Aqua. Ecol., 35: 245-255.

Szefer, P., J. Rokicki, K. Frelek and M. Malinga, 1998. Bioaccumulation of selected trace element in lung nematode, *Pseudalius inflexus*, of harbor (*Phococena phocoena*) in a polish zone of Baltic Sea. Sci. Total Environ., 220: 19-24.

Tariq, J., M. Jaffer and M. Ashraf, 1993. Heavy metal concentration in fish, shrimp, seaweed, sediment and water from Arabian Sea, Pakistan. Mar. Pollut. Bull., 26: 644.

Tenora, F., V. Barusi, S. Kracmar, J. Dvoracek and J. Srnkova, 1999. Parallel analysis of some heavy metals concentration in the *Anguillicola crassus* (Nematode) and the European eel *Anguilla anguilla* (Osteichthyes). Helminthologia, 362: 79-81

Tenora, F., V. Barus, S. Kracmar and J. Dvoracek, 2000. Concentration of some heavy metals in *Ligula intestinalis* plerocercids (Cestoda) and *Philomera ovata* (Nematode) compared to some their hosts (Osteichthyes). Helminthologia, 37: 15-18.

Trucekova, L., V. Hanzelova, M. Spakulova, 2002. Concentration of heavy metals and its endoparasites in the polluted water reservoir in Eastern Slovakia. Helminthologia, 39: 1-23.

Ucman, E., M. Vavrova, S. Zima, P. Kooinek, J. Pavelka, H. Zlamalova and H. Gargo-Ova, 2001. Studies on the transfer of harmful substances from feed chicken tissues. Canadian J. Anal. Sci. Spectrum, 46: 89.

Voegborlo, R.B., A.M.E. Methnani and M.Z. Abedin, 1999. Mercury, cadmium and lead content of canned tuna fish. Food Chem., 67: 341.