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Perna perna (LINNAEUS, 1758) MUSSELS IRRADIATED BY ^{60}CO GAMMA RAYS CYTOTOXICITY EVALUATION

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ABSTRACT

The aim of the present work was the study of ionizing radiation effects on aquatic biota regarding the location of nuclear facilities nearby coastal areas assuming the risk of leaks and nuclear accidents. Bivalve mollusks have been widely used in the monitoring of aquatic environment studies mainly for their sessile habit and pollutants bioconcentration ability. So marine mussel *Perna perna* (Bivalvia: Mytilidae) was used as organism test in this study. The study of radioactive toxicity was performed by cytotoxicity test exposing the organisms to 11Gy gamma radiation dose. After radiation the neutral red retention assay evaluated the lysosomal membrane integrity in the mussel hemocytes. 50% lethal dose assay (LD50) of gamma radiation on *Perna perna* mussels was carried out by exposure the organisms to ^{60}Co gamma rays at doses ranging from 0 to 3000 Gy. The result of gamma radiation LD50 for these mussels was 1068 Gy and the neutral red retention time of irradiated organisms was about 47% lower than the control, non irradiated organisms. With the obtained results is expected to contribute in the study to identify the range of ionizing radiation doses which can cause toxic effects in marine invertebrates.

1. INTRODUCTION

The human protection against radiation can be defined as the set of measures that aim to protect the human being and his descendants against possible unwanted effects caused by ionizing radiation [1]. Such measures are proposed by the International Commission on Radiological Protection - ICRP, which are standard procedures internationally adopted. Similarly, the environmental protection against radiation can be defined as a set of measures aimed to protect the biota from unwanted effects of ionizing radiation. However, there are no standard criteria proposed specifically for the environmental protection [2, 3].

The existing procedures to protect the environment against radiation are performed considering only the release of effluents and, based on a paradigm proposed by ICRP [3]: "The commission believes that the standard of environmental control needed to protect man to the degree currently thought desirable will ensure that other species are not put at risk (ICRP-26 1977)". Such statement has been discussed by many authors [2-7]. In addition, the

ICRP stated: “Occasionally, individual members of non-human species might be hammed, but not to the extent of endangering whole species or creating imbalance between species [3].”

Since the ‘70s, the concepts and parameters to be followed for environmental protection against radiation are evolving, but especially after the ECO-92 (United Nations Conference on Environment and Development – UNCED), the concept of environmental protection became in evidence.

The ECO-92 proposed specific recommendations on radioactive waste and the environment, in order to support the efforts of the IAEA to develop and promulgate security and standards, guidelines and codes of practice to be accepted internationally in the management and disposal waste of radioactive to the environment. The ECO-92 also pointed out that the protection of the environment should be made in terms of preservation of biological and genetic diversity and conservation of living resources and habitats [8].

Due to the fact that nuclear facilities are located in the Brazilian coast, the radiological impact assessment on coastal ecosystems and aquatic biota is, in general, of the utmost importance.

The need to establish systems that demonstrate that the biota this protected against unwanted effects of ionizing radiation is required internationally [3, 8-11]. A variety of scenarios may require a radioecological assessment of non-human biota. For example, this may occur in the context of environmental site remediation [12], radioactive waste disposal [13], or nuclear power plant accidents [14].

According to Pentreath [5], the environment protection should be clearly demonstrated, independent of the presence or absence of humans. Therefore, the purpose of this paper was to study the biological effects of ionizing radiation on aquatic biota. In our study, 50% lethal dose (LD50) of ^{60}Co gamma radiation on mussel was determined and the radiation cytotoxicity was evaluated by the neutral red retention time on irradiated organisms.

2. METODOLOGY

2.1. Organism test and sea water

The organisms as well as the water used in the acute toxicity and cytotoxicity tests were collected from an unpolluted area at Cocanha beach – Caraguatatuba, SP. The water was packed in barrels of 50L, transported and kept at a temperature of 22±2°C. The used organisms test were *Perna perna* mussels with 4-5cm long and were kept at laboratory for 1 to 3 days in static sea water tanks. *Perna perna* marine mussel is commonly used as bioindicator.

2.2. Gamma radiation lethal dose (LD50)

Gamma radiation LD_{50} is the radiation dose which provokes the death of 50% of the organism population in the assay.

This test was performed in triplicate. For each radiation dose 5 organisms in polypropylene bottles containing 750 ml marine water was used. These bottles were irradiated by gamma rays from ^{60}Co source of Gammacell 200 at 0, 250, 500, 750, 1000, 1500, 2000, 2500 and 3000 Gy doses and 1.64 kGy.h^{-1} dose rate (Fig. 1 and 2).

The control bottle was not irradiated (0 Gy) and the mortality of total organisms were observed after 72h.

Figure 1: *Perna perna* mussels in polypropylene bottles for irradiation

Figure 2: *Perna perna* mussels inside Gammacell 200

2.3. Neutral red retention assay (NRRA)

The neutral red retention assay was used to verify the ionizing radiation effect on the hemolymph hemocytes lysosomal membrane of *Perna perna* mussels. The NRRA method followed the protocol proposed by Lowe and Fossato [15]. The test is performed by the evaluation of lysosome membrane integrity in the hemocytes. The organisms were irradiated with 11 Gy and the results were obtained 24, 48 and 72h after irradiation and the test endpoint was when is observed the evident dye loss in at least 50% of the hemocytes.

The mussels (n=15) were placed in polypropylene bottles containing 750 ml marine water and were irradiated by ^{60}Co gamma rays at 11 Gy dose, and after 24, 48 and 72h the NRRA was applied. Fifteen slides were used for control and irradiated organisms.

The mussel hemolymph was extracted from the posterior adductor muscle and an aliquot of 40µL of sample containing hemocytes was placed carefully on top of the histological slide prepared in advance with poly-L-lysine covering.

Slides were left on a rack in a light-proof humidity chamber during the assay. After 15 min the excess solution was carefully tipped off and 40µL of neutral red working solution were added. Slides were thereafter examined systematically under an optical microscope at 15 min intervals to determine the evidence of dye loss from the lysosomes to the cytosol of the hemocytes. It was observed a decrease in the neutral red retention time in comparison to the control, non-irradiated organisms.

3. RESULTS and DISCUSSION

The results of radiation acute ecotoxicity test are shown in the Table 1.

Table 1. Results of *Perna perna* lethality percentage in the radiation acute toxicity test.

Gamma radiation doses (Gy)	Lethality (%)		
	Assay 1	Assay 2	Assay 3
0	0	0	0
250	0	0	0
500	0	0	0
750	0	0	0
1000	40	40	60
1500	100	80	100
2000	100	100	100
2500	100	100	100
3000	100	100	100

The software *Trimmed Spearman-Karber* was used to calculate the gamma radiation lethal dose (LD50). LD50 is the gamma radiation dose which provokes 50% lethality of *Perna perna* mussels after irradiation.

The dose response curve of LD50 assay is shown in Fig.3.

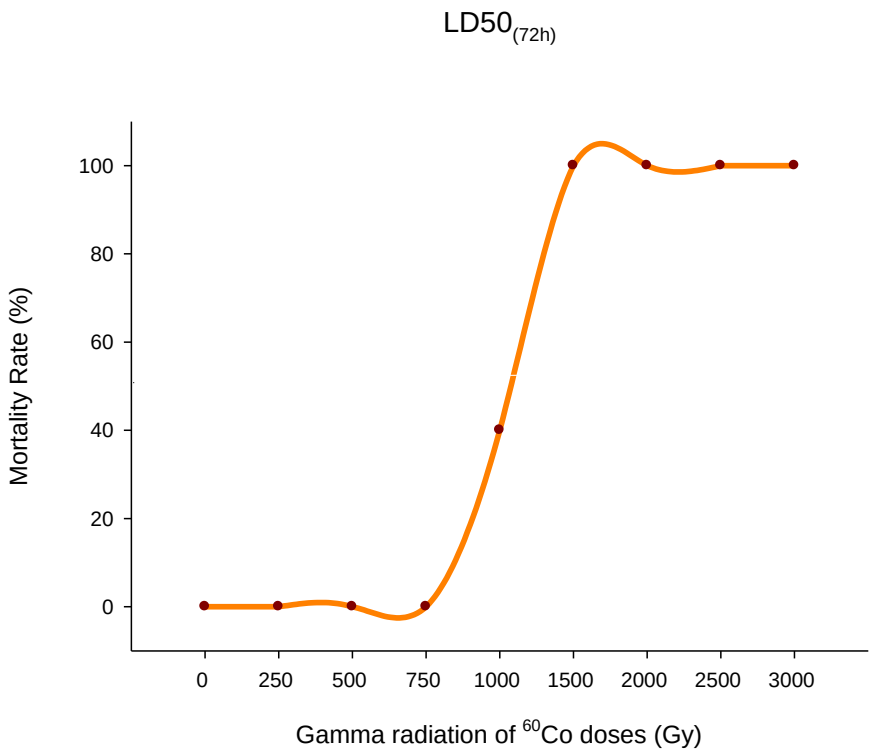

Figure 3. Dose response curve of gamma radiation LD50 assay for *Perna perna* mussels.

The LD50 value for gamma radiation acute ecotoxicity tests in the *Perna perna* mussels was 1068 Gy.

With obtained LD50 was determined the radiation dose to be used in the cytotoxicity test. There is no data about this in the literature, so the experiment was started with pre test utilizing a dose with a hundred times lower than LD50.

The cytotoxicity test of irradiated organisms was evaluated by the retention time of neutral red dye. The results from the statistics analysis carried out with the student's T-test are shown in Table 3 and Fig. 4.

Table 2. Results of neutral red retention time in the gamma radiation cytotoxicity assay to *Perna perna* mussels.

Irradiation dose (Gy)	Neutral red retention time (min) after irradiation					
	24h		48h		72h	
	Medium ± SD*	VC**(%)	Medium ± SD*	VC**(%)	Medium ± SD*	VC** (%)
0	56±13	23	53±11	21	50±13	26
11	29±15	50	30±15	50	28±16	57

*SD – standard deviation, **VC – variation coefficient

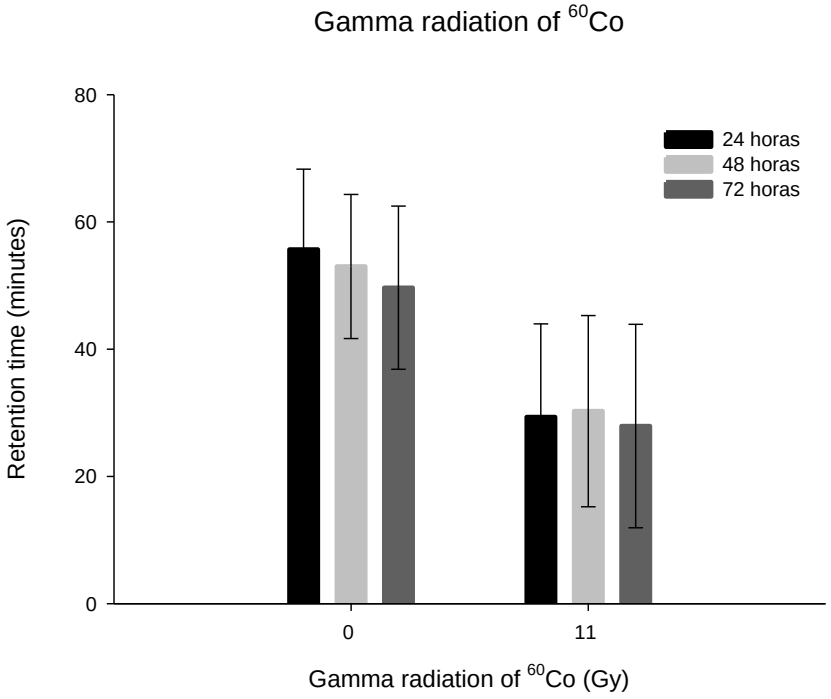

Figure 4. Neutral red retention time comparison of irradiated and non irradiated *Perna perna* mussels

4. CONCLUSIONS

The ^{60}Co gamma radiation lethal dose for *Perna perna* mussels was 1068 Gy.

After gamma irradiation of mussels at 11 Gy dose it was observed a decrease of 47% in the neutral red retention time in the hemocytes lysosomes. These results indicate that ionizing radiation, and specifically gamma radiation causes a significant cellular stress in the *Perna perna* mussels, causing lysosome membrane destabilization.

With the obtained results is expected to contribute in the identification of the ionizing radiation doses range which can cause toxic effects in marine invertebrates to the radioecotoxicology studies.

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