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A COMPARATIVE STUDY ON GENOTOXIC EFFECTS IN OLIGOCHAETA FROM AREAS WITH ELEVATED NATURAL AND ARTIFICIAL RADIOACTIVITY

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Abstract. A comparative study on genotoxic effects in worm *gen. Nais* (Oligochaeta) from two selected areas of elevated natural and artificial radioactivity has been carried out. The areas considered were a lake in the Chernobyl NPP area in Ukraine and a spring-created pond in the Ikaria island, in the eastern Aegean sea, Greece. The chromosome aberrations recorded in worm *Nais pseudobtusa* from the lake in Chernobyl were attributed to β- and γ-ionising radiation. Unlike these findings, the chromosome aberrations and the pycnotic nuclei observed in worm *Nais communis* from the spring-pond in the Ikaria island were probably caused by the combined action of low pH, high water temperature and natural radionuclides as heavy metals or/and micro-aggregated one.

Keywords: radiation effects, chromosome aberrations, the Ikaria island, Chernobyl, radioactivity in lakes.

AIMS AND BACKGROUND

Comparative genotoxic effects of natural and artificial radionuclides on native populations of organisms in environmental conditions can provide important information for understanding the action mechanisms of deleterious factors and their contributions to the total damage observed.

The cytogenetic effects of radioactive and conventional pollution as they are recorded in organisms of natural ecosystem and the apportionment of causes to each kind of pollutants is a relative new field in radioecological research. There

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is limited evidence on field observations in international literature; even there is a lot of evidence in concerning laboratory experiments. This research field has been developed mainly after the Chernobyl nuclear accident through studies of radiation effects in natural populations of aquatic organisms in the wide area of impact[1-6].

The aim of this study is to compare the impact of elevated natural and artificial ionising radiation on worm populations *gen. Nais* (Naididae, Oligochaeta) in the Ikaria island and in the Chernobyl NPP zone, looking at the cytogenetic level of the sampled specimens.

The Ikaria island. The Ikaria island (37°59′ N, 22°58′ E – an area of 267 km^2 in the eastern Aegean sea) has been shown in several research studies in the marine environment of Greece to have some areas with elevated natural background radiation due to geological origin[7]. These areas, mainly cited in the neighbourhood of several metallic or/and thermo-metallic springs spread in the Island, present elevated concentrations of natural alpha and gamma-emitters in the abiotic components of the environment (e. g. the reported concentrations in soil, ores, mud, etc. are for ^{238}U up to 1050 Bq kg^{-1}, ^{226}Ra up to 760 Bq kg^{-1}, ^{228}Ra up to 260 Bq kg^{-1}, ^{228}Th up to 70 Bq kg^{-1}, ^{232}Th up to 300 Bq kg^{-1}, ^{40}K up to 2700 Bq kg^{-1}). Besides, elevated concentrations of ^{222}Rn (110-970 Bq l^{-1} for inland waters and 2. 7- 35 Bq l^{-1} for coastal seawater) were reported in the above cited studies. Concerning the local aquatic organisms, they have been found to present higher respective concentrations if compared to those from other areas in Greece[8].

In the areas of springs, the inhabited biota chronically exposed at these levels are also exposed to higher temperatures especially during the cold period (up to 26 and 43°C during the cold period and warm period, respectively – supported measurements in the wide area of the Island for the present study). The measured gamma dose rates in the vicinity of the springs range as 0.15-0.21 μGy h^{-1} and in the control areas as ≤ 0.5-0.12 μGy h^{-1}, whereas the mean value for the total measured area is 0.14 μGy h^{-1} (Fig. 1). The reported mean value for Greece (terrestrial and coastal sediment surface) is 0.08 μGy h^{-1} (Ref. 7).

Among the several metallic springs, an inland spring with its sources in a rocky hill (mountainous area in the southern part of the Island) is considered in the present study (Fig. 1). The spring water on the feet of the hill has created a shallow pond. The gamma dose rate at 1 m above the ground in the pond surrounding area was 0.21 μGy h^{-1}, whereas the respective value for the bottom sediments of the pond was in the same range. Nevertheless, a maximum of 1.08 μGy h^{-1} was measured as a spot value attributed to the influence of ore transported material[8]. The concentrations of the natural alpha and gamma emitters in the nearby mud and the bottom sediments of the spring-pond were found to the upper part of the range reported above for the wide area of the Island. It is of meaning that the respective concentrations of ^{238}U were in the levels of the reported maximum (500-1000 Bq kg^{-1}) The supporting field parameters of pH and temperature were 5.3-6.0 and

33°C, respectively. The pH value in the starting point of the spring (the outflow point in the hill) was 5.3 and the temperature of the spring water in the outflow point on the hill was 29°C.

The Chernobyl lake. In order to compare the effects of natural radioactivity to those of artificial impact, the findings from a lake located in the area of the 'red forest' in the Chernobyl zone (near to the NPP) have been placed near. The gamma and beta dose rates in the bottom sediments of this lake were 9.0 and 5.0 μGy h^{-1}, respectively. The pH was 6.5 and the water temperature was 17°C (Ref. 4).

EXPERIMENTAL

To determine the natural gamma-radiation status in the Ikaria island, gamma-radiometry has been applied in a network of 300 km (mean value recorded every km, Fig. 1). A car-borne scintillometer with a 2″ × 2″ NaI (Tl) cylindrical detector was used for the radiometry (sensitivity 1 cpm per 3.5×10^{-3} μR h^{-1} for ^{226}Ra at 1 m about ground – the original calibration of the instrument).

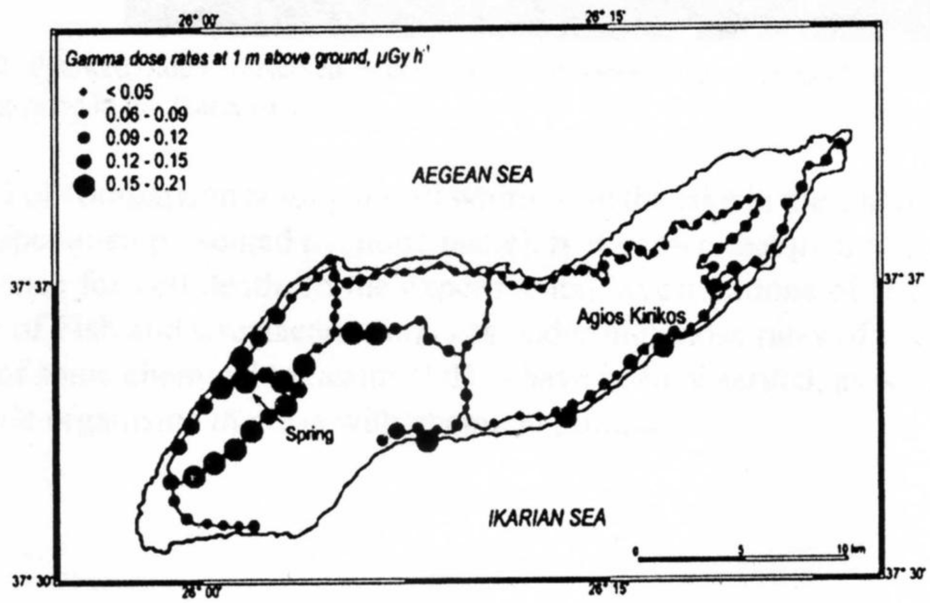

Fig. 1. Map of the Ikaria island with the site of the spring-pond and the gamma-radiometry network

During an expedition on July 2003 in the Ikaria island, samples of macrophytes with worm Nais communis on them were collected from the small pond described above. Spring water and bottom sediments were also collected for supporting measurements and *in situ* hydrochemical parameters were also recorded. Supported field parameters were also recorded in the wide area of the Island during this time and data form previous studies were also used as conjoined to the findings of the present study[5]. Concerning the lake in the 'red forest', worm *Nais pseudobtusa* were collected following the above protocol in September 1995. In both cases worm were sampled from the surface of the collected macroptytes by sequential rinsing with water in a glass pot. The worm were fixed in a 3:1 mixture of ethyl alcohol and glacial acetic acid and stained with 1% aceto-orcein. The specimens were squashed in 60% lactic acid for cytogenetic analysis. Chromosome aberrations were scored at the anaphase-telophase stage of mitosis in worm reproduction by paratomic division.

RESULTS AND DISCUSSION

The frequencies of induction of chromosome aberrations are given in Table 1 and the types of the observed aberrations – in Table 2. One can note that the percentage of aberrant cells in both cases is similar. Nevertheless, there are some observations, which differentiate the two cases. A very small number of dividing cells in worm from the spring-pond was counted (150 divided cells of 23 specimens, whereas in the Chernobyl lake 574 cells of 30 specimens). Besides, among the 23 specimens with divided cells, 5 specimens were found to have local sites with pycnotic nuclei (Fig. 2).

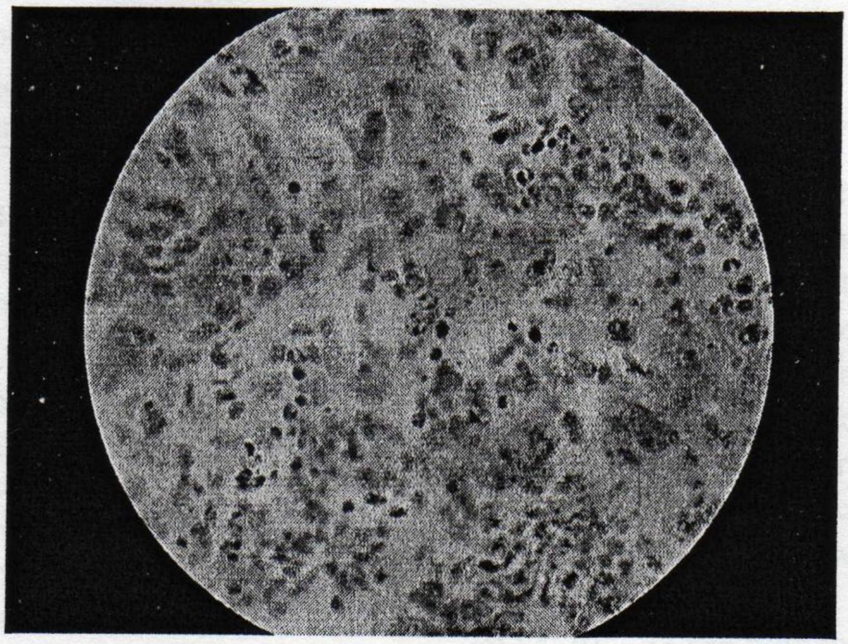

Fig. 2. Pycnotic nuclei observed in cells of *Nais communis* (gen. Oligochaeta) sampled from the spring-pond in the Ikaria island

For comparison among the 30 worm from the lake in the Chernobyl zone only one specimen presented pycnotic nuclei. It is known that pycnotic nuclei provide evidence for cell death. In the experimental investigations of IBSS, pycnosis in cells of Fish and Crustacean embryos under high dose rates of ionising radiation and of some chemical toxicants (DDT) have been observed, as well as, in cells of marine organisms in areas with chemical pollution[10].

Table 1. Frequency of chromosome aberrations in worm from the two sampling areas

Species	Location	Number of worm	Number of cells counted	Cells with aberrations	
				number	%
Nais pseudobtusa	lake Chernobyl zone	30	574	46	8.4
Nais communis	metallic spring the Ikaria island	23	150	12	8.0

In the IBSS several studies have been performed under Laboratory and *in situ* conditions on the radiation cytogenetic effects in aquatic organisms, e.g. Crustacean and Fish[11-13]. It has been shown that under the influence of ionising radiation (beta and gamma) the distributions of chromosome aberrations between cells are better described by the Poisson than the geometric distribution (i.e. the x^2 is lower for Poisson than that of geometric distribution), whereas the converse is the case for exposure to chemical mutagens. Under the combined influence of the two factors, the observed distribution is closer to the Poisson than the geometric distribution if the ionising radiation is more effective, but closer to the geometric if the two factors are of comparable effectiveness.

In terms of the types of the observed aberrations (Table 2), one can note that in both cases, the cells with single bridges show the maximum percentage among the aberrant ones, but higher is in the case of *Nais pseudobtusa* from the Chernobyl lake. Considering the other types, there are quantitative and qualitative differences between the aberrant cells from the two sampling areas.

Table 2. Types of chromosome aberrations in worm from the two sampling areas (%)

Types of chromosome aberrations	*Nais pseudobdusa*	*Nais communis*
Single fragment	34.1	18.8
Twin fragment	5.5	12.5
Chromatid ring	–	6.2
Chromatid ring and fragment	–	6.2
Single bridge	53.9	43.8
Single bridge with fragment	4.7	–
Single bridge with twin fragment	–	12.5
Twin bridge	1.8	–

The distributions of chromosome aberrations in cells of the worm from the two locations are presented in Table 3. According to the statistics, the distribution of chromosome aberrations in cells of *Nais pseudobtusa* from the Chernobyl lake fits more closely the Poisson distribution ($\chi^2 = 0.42$ for the Poisson compared to $\chi^2 = 1.98$ for the geometric one). In this case, the observed effects are mainly attributed to β- and γ-ionising radiation. In the worm *Nais communis* from the spring in Ikaria the distribution of chromosome aberrations in cells seems to conform to the geometric distribution ($\chi^2 = 0.41$ for the geometric compared to $\chi^2 = 0.76$ for the Poisson). Therefore, the statistics of the distribution of aberrations recorded in the worm under the influence of the metallic spring indicates chemical effects.

Table 3. Distribution of chromosome aberrations in cells of worm from the two sampling areas

Species	Location	Chromosome aberrations in a cell	Frequencies		
			observed	expected for the Poisson distribution	expected for geometric distribution
Nais pseudobtusa	lake Chernobyl zone	0	528	528.9	530.4
		1	45	43.3	40.3
		2	1	1.8	3.1
				$\chi^2 = 0.42$	$\chi^2 = 1.98$
Nais communis	metallic spring the Ikaria island	0	138	134.8	135.6
		1	9	14.4	13.0
		2	2	0.8	1.2
		3	1	0.03	0.1
				$\chi^2 = 0.76$	$\chi^2 = 0.41$

The sum of the observations of the genotoxic effects on the worm from the spring-pond in the Ikaria island is as follows: (a) a small number of dividing cells among the collected specimens, (b) a high percentage of chromosome aberrations, (c) some specimens with pycnotic nuclei in local sites of cell observations, and (d) better fitting of chromosome aberrations to the geometric distribution than the Poisson one.

The results obtained can be interpreted by supposing the following assumptions: According to published findings[14-19] uranium and transuranium elements can form micro-aggregations in tissues of aquatic organisms. Inside the organisms, in the micro-vicinities of those micro-aggregations, the size of which varies up to 200 microns, the local concentrations of radionuclides and the consequent dose rates of α-radiation can exceed the mean up to three orders of magnitude[20]. Besides, as it is known, in the aquatic systems, the actinides are readily absorbed on the surfaces of plants and small animals[21], whereas acid pH have been found to increase the distribution of ^{241}Am in freshwater snails[22]. Nevertheless, one can suppose that as the solubility of uranium increases in acid pH aquifers, the re-dissolution from the bottom sediments is expected to enhance with a consequent increase of uranium bioavailability (pH in the spring-pond 5.3-6). Nevertheless, low pH and high water temperature (33°C) could cause damage by themselves or/and be synergetic factors. It is noteworthy that chromosome aberrations conformed to geometric distribution pattern have been recorded in *Polychaeta* and *Gammaridae* from the coastal areas in the outflow of another metallic spring in the island (the spring called Therma) by the authors[5].

Therefore, it can be suggested that the combined effect of low pH, high temperature and heavy natural radionuclides (possibility, in the form of micro-aggregations as α- emitted radionuclides and as chemical toxicants – heavy metals) were observed in the cellular level of the sampled *Oligochaeta* inhabited the spring-pond.

CONCLUSIONS

The observed genotoxic effects in worm (Oligochaeta) sampled from the spring-created pond in Ikaria could be attributed to the combined action of low pH, high temperature and heavy natural radionuclides as toxic metals or/and probably in form of micro-aggregations of them (a-emitters), with a subsequent high dose rate exposure. The respective chromosome aberrations in worm from the lake in Chernobyl zone seem to be mainly induced by β- and γ-ionising radiation.

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