Micro-organisms and Divers Exposure to Radioactivity in Spent Fuel Pools at Nuclear Power Plants

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Abstract

Many nuclear power plants (NPPs) around the world are in the process of extending their lifespan from 40 to 60 years of operation. The NPP; Angra 1 (Brazil) has performed a thorough evaluation of its Life Extension Engineering project. In this context, the spent fuel pool (SFP) was one of the areas studied in order to demonstrate the plants integrity for a life extension. Micro-organisms growing on the liner of the fuel transfer channel (FTC) and SFP can form a film of bacteria, which is highly resistant to radiation. This paper aims to compare the micro-organisms found in NPP Angra 1 with those reported to other NPPs and also relates their occurrence with the radiation levels at the sites. It also compares diver’s exposure to radioactivity during underwater activities in the SFP. Fourteen samples were collected on the surface of the liners of the FTC, the SFP and the drains within the fuel building (FB) of Angra 1. For the identification of the micro-organisms, a metagenomics analysis was performed by random sequencing (Shotgun) and the use of Ion Torrent PGM Sequencer. Twelve micro-organisms phyla were identified; Acidobacteria, Actinobacteria, Bacteroidetes, Chlamydiae, Chlorobi, Chloroflexi, Cyanobacteria, Deinococcus-Thermus, Firmicutes, Planctomycetes, Proteobacteria, and Verrucomicrobia as well as organisms not classified. In the SFP of Angra 1, the bacteria survived the exposure to a radiation of 0.416 Gy/h (high radiation). Deinococcus-thermus, bacteria identified in Angra 1, has resisted an exposure to 30,000 Gy/h in another plant.

1. INTRODUCTION

Many NPPs around the world have reported the proliferation of algae, fungi and bacteria on the surface of the SFP. The biofilms formed by these micro-organisms may or may not compromise the surfaces (IAEA, 1997) of the pools. In 1990 at the Jaslovské Bohunice NPP (Slovakia Republic), the growth of psychrophilic and mesophilic bacteria and micromycetes in the SFP were detected. The phenomena resulted in the partial decrease in water clarity and the flocculation of the cells present in pool water. In this particular case the biomass growth occurred mainly due to favorable conditions in the pool, such as low water flow and temperature (IAEA, 1997). The Pickering NPP (Canada) reported the frequent clogging of the SFP purification filters caused by the excessive growth of micro-organisms. The analyses of the filters indicated the presence of Actinomycetes (gram-positive and aerobic) and Polysaccharides. At that time, to eliminate the biofilm, the walls and the bottom of SFP were brushed and hydrogen peroxide was added to the water at a concentration of 150 ppm (IAEA, 1997). A microbiological study based on standard culture methods and sequencing of the gene encoding 16S ribosomal RNA (16S rRNA) conducted at the Confrentes NPP in Spain, detected the formation of biofilms composed of various species, despite the oligotrophic conditions and radioactive water (Sarró et al., 2005). For that study, austenitic stainless steel and titanium (99.9) plates were immersed in the SFP in static and dynamic conditions. After 34 months of immersion, a biofilm formation was observed and then confirmed by epifluorescence electron microscopy. From those samples, fifty-seven different bacteria were identified belonging to α, β and γ Proteobacteria Phylum, Firmicutes and Actinobacteria (Gram-positive bacteria). The radioactivity of the biofilm was measured using γ-ray Spectrometry, revealing that the biofilms were able to retain Cobalt 60 radionuclides (Sarró et al., 2005).

The experience of global NPP's confirms the identification of micro-organisms and biofilm formation in fuel pools, even with high quality parameters regarding the physical, chemical and radiometric characteristics of the water in the pool. Although up to now they do not compromise the integrity and the technical environment, they can potentially cause changes in the water quality and at extreme cases superficial corrosion. These facts highlight the importance of the present work that aims to identify the bacteria growing at the NPP Angra 1 (Brazil) and compares their occurrence to other micro-organisms thriving in SFP´s walls.

2. METHODOLOGY

Fourteen samples were collected on the surface of the liners of the FTC and the SFP as well as in and around the drains of the fuel building (FB) of the NPP Angra 1. For the identification of the micro-organisms, a metagenomics analysis was performed by random sequencing (Shotgun) and the use of Ion Torrent PGM Sequencer. The details of the methodology of collection and analysis of the samples can be verified at Almeida (2016) e Almeida et al. (2016).

The information on the exposure of divers to radiation was obtained from the Underwater Construction Corporation (UCC) data base (personal communication).

3. RESULTS AND DISCUSSION

A total of twelve phyla were identified as growing on the liner of the FTC and the SFP as well as in and around the drains of the FB. The micro-organisms were identified as; Acidobacteria, Actinobacteria, Bacteroidetes, Chlamydiae, Chlorobi, Chloroflexi; Cyanobacteria, Deinococcus-Thermus, Firmicutes, Planctomycetes, Proteobacteria, Verrucomicrobia (Table I). The study of Ruiz et al. (2016) isolated eighty seven bacterial morphotypes; 18 from a high background radiation area (2.9 µGy/h), 25 of background radiation (0.45µGy/h), 20 from a low background radiation (0.1µGy/h) and 24 on a control area (environmental background radiation of 0.03-0.05µGy/h). The study determined that the bacteria belong mainly to the Group of Bacillus-Firmicutes (41 morphotypes) and Actinobacteria (27), less frequently Gammaproteobacteria (3), Flavobacteria (2) and Betaproteobacteria (1). The lowest species richness was found in areas of high background radiation (Table I). All bacteria identified at the SFP of Angra 1 were also found in samples of most SFP drains. It can be assumed that the bacteria cells traveled the 12 meters of water column. In this way, it can be suggested that all of them are resistant to high radiation that survived exposure to 894 irradiated fuel elements stored with high activity (416,000 µSv/h = 0.416 Gy/h) located in the SFP.

The radiation exposure and the micro-organisms found in Angra 1 and in the world NPPs are summarized in Table I. Among the 12 phyla (excepting the bacteria Chlamydia, Chlorobi, Chloroflexi, and Firmicutes), only eight of them have been found in the areas Chernobyl accident by (Ragon et al. 2014). Ruiz et al., 2016, mentions that Flavobacteria Firmicutes (Bacillus) and Proteobacteria were identified in the areas after the Chernobyl accident resisting an exhibition of 4,000 Gy/h. Among the many articles published about the accidents of the NPP at Chernobyl in 1986 and Fukushima in 2011, only three of them, referring to Chernobyl, contained information about bacteria (Ruiz et al., 2016; Møller et al., 2013 and Ragon et al., 2014). The Chernobyl accident has long been considered a long-term experiment on the effects of ionizing radiation exposure to the ecosystem level. Although studies of these effects on plants and animals are abundant, the study of how the Chernobyl radiation levels affect microbial communities is virtually non-existent, except for a few reports on human pathogens or micro-organisms of the soil (Ragon et al. 2014). The authors found a positive correlation between the increased radiation exposure and increased rates of mutation in Actinobacteria found in samples of Chernobyl and Northern Ireland. The Actinobacteria, identified in Angra 1 was also present in the areas post-Chernobyl accident (Ruiz et al., 2016), but resisting an exhibition well in excess of 4,000 Gy/h and in the NPP of Confrentes in Valencia in Spain (Sarró et al., 2005 and Chicote et al., 2004), and Pickering in Canada, (IAEA, 1997) these values have not been mentioned by radiation exposure. Cyanobacteria were also observed in areas post-Chernobyl accident (Ragon et al., 2014), resisting a 0.000025Gy/h, well below the Angra 1, however, these bacteria resisted an exhibition of 4,000 Gy/h (Billi et al., 2000).

Table I. Bacteria found on NPP of Angra 1 and others NPP in the world.

Nuclear Power Plants	**Present study**	**Ragon et al. (2014)**	**Ruiz et al. (2016)**	**Sarro et al. (2005)**	**Chicote et al. (2004)**	**IAEA (1997) "Canada"**	**Møller & Mousseau (2013)**	**IAEA(1997) "Slovakia"**
Activity (Gy/h)	**0.41**	**25×10^{-6}**	**4,000**	**NM**	**NM**	**NM**	**NM**	**NM**
Acidobacteria	x	x						
Actinobacteria	x	x	x	x	x	x	Kineococcus	
Alfa proteobacteria	x	x					Methylo bacterium; Rubrobacter	
Bacteroidetes	x	x	flavo bacteria					
Beta proteobacteria	x	x			x			
Chlamydiae	x							
Clorobi	x							
Cloroflexi	x							
Cyanobacteria	x	x					Chrooco ccidiopsis	
Deinococcus-thermus	x	x						
Delta proteobacteria	x	x						
Firmicutes	x		Bacillus	x	Staphylococcus		Paenibacillus amyloliticus; Bacillus subtilis	
Gamma proteobacteria	x	x					Acineto bacter radio resistens; Azotobacter; Escherichia coli	
Planctomycetes	x	x						
Verrucomicrobia		x						
indeterminate bacteria	x	x						
Achromobacter xylosoxidans	x		Proteo bacteria	57 bacteria: actino bacteridae and proteo bacteria				
Others groups	x				Trichocomaceae	Poli-ssaca rides	Desulfuro coccus; Locurio rósea; Radio tolerans;	Maso philes; micro mycetes ;Psychro

							Thermo coccus	philes

NM – value not mentioned at the publication

Until recently, it was not speculated that micro-organisms could grow at the SFP at Angra 1. The work of Almeida (2016), pioneered the detection of micro-organisms growing in the SFP of Angra 1. Almeida et al. (2016) reported the isolation, growth on the culture medium and subsequently identification by 16S rRNA gene (sequenced by Sanger method) of the bacteria *Achromobacter xylosoxidans*. As shown in Table II many micro-organisms survive high and prolonged exposure to radioactivity in the SFP. The bacteria Deinococcus-Thermus, identified in Angra 1 and Chernobyl (Ragon et al., 2014), resisted an exhibition of 30,000 Gy/h (Rainey et al., 2005). The table also indicates that divers performing activities in SFP do not absorb all the radiation to which they are exposed, indicating that the protection used and treated pool water act effectively to protect individuals. This fact is of fundamental importance since doses of only 2 Gy/h can be lethal for humans.

Table II. Human and micro-organisms exposure to ionizing radiation

Ionizing Radiation Resistance	**Dose Gy/h**	**Note**
Occupational exposure (outside de pool) of Angra 1 NPP	0.02	Employee/year (Eletronuclear, 2017)
Occupational exposure (outside de pool) in NPP in the world	0.05	Employee/year (Eletronuclear, 2017)
Occupational Angra 1 NPP	0.014	Employee/23 years (Almeida, 2016)[1]
Occupational Angra 1 NPP (diver)	0.00049	Diver/day (Almeida, 2002)[2]
Occupational Other NPP (diver)	0.01	/Diver (UCC, 2017)[3]
Occupational NPP Fort St Vrain NPP (diver)	0.02	/Diver (UCC, 2017)[4]
Mortal during exhibition	1000	Human (Leitão, 2017)
Death in 48 h	50 a 100	
Death from 5 to 10 days	6 a 50	
Death from 10 to 30 days	2 a 6	
Deinococcus-Thermus	30,000	Bacteria (Rainey et al., 2005)
Cianobacteria	4,000	Bacteria (Billi et al., 2000)
Acidobacteria	0.416	Bacteria in SFP - Supercompact racks of Angra 1, 894 irradiated fuel elements. Almeida (2016) and Almeida et al. (2016).
Actinobacteria		
Alphaproteobacteria		
Bacteroidetes		
Betaproteobacteria		
Chlamydiae		
Clorobi		
Cloroflexi		
Cyanobacteria		
Deinococcus-Thermus		
Deltaproteobacteria		
Firmicutes		

Gammaproteobacteria		
Planctomycetes		
Verrucomicrobia		

1- Ocupacional exposure after 23 years of work in Angra 1 was 0.01495 Gy occupational dose allowed/ 23 years would be of 0.46 Gy.

2- Occupational exposure within the STC, by a nuclear diver, exposed in 2:20 = during the underwater maintenance fuel transfer system of Angra 1, the occupational dose 0.00049 allowed/day would be 0.001 Gy.

3- Occupational exposure by UCC´s nuclear divers during Project Spotlight: BWR Steam Dryer Segmentation

-Dose rates 0.05 to 0.14 Gy contact
-Shielding - S.S. Plate and Powder Tungsten encased in S.S. Plate
-295 dives, 728 h in-water time, 30 days on 24/7 schedule with 44 divers
-Estimated 0.47 man Gy - 0.01 Gy/diver

4- Occupational exposure by UCC´s nuclear divers during Underwater Plasma Cutting in NPP Fort St Vrain Decommissioning

-18 months, 22 nuclear divers, 1,650 dives, 3,400 in-water hours, 200 tons of material removed
-Contact dose 0.4 to 0.7 Gy/Working dose 0.00016 Gy/h;
-Total exposure 0.51 Gy or approximately 0.02 Gy per diver

The radiation dose potentially received by the micro-organisms in the pool is much higher than the dose received by the SFP workers. Table III presents the difference in exposure of human and micro-organisms to ionizing radiation in Angra 1. The occupational exposure report at the Angra 1 shows that after 23 years working in the vicinity of the SFP and the FTC, workers received a low dose of radioactivity 14.95 mSv. This is due to the design of the SFP, where the depth of approximately 12 meters of water column eliminates the possibility of radiation exposure at the edge of the pool. The effective preventive cautions safeguarded the workers from contamination in case of contact with the pool water by the use of appropriate clothing and the control on the exclusion of foreign material to the work area. The proper inventory and disposal of all material and tooling used for any task prevents their misplacement therefore reducing the risk of falling into the pool and cause irreparable damage to systems and the fuel. This data indicates that the radiation detected during the analyses is restricted to the pool.

The sample collected in this study (SWAB) did not show significant levels of radionuclides, answering to all criteria to remove and transport material originated at Angra 1. It was not possible to observe evidence of increasing levels of radiation in the pool wall from the micro-organisms through the formation of biofilms or any of bio-corrosion. In the study of Sarró et al. (2005), 57 bacteria were identified belonging to the groups; Proteobacteria, Firmicutes and Actinobacteria, revealing that the biofilms were able to secure, especially Cobalt 60 radionuclides. This fact probably is justified by the fixed (non-transferable) contamination of radionuclides bodies of evidence of austenitic stainless steel and titanium (99.9), after 34 months immersed in the pool.

Table III. Occupational Radiation Exposure Report at the Angra 1 NPP.

Period of Exposure	Dose mSv	Dosimeter Type
From Nov 1st, 1992 to Dec 31st, 1992	3.60	Dosimeter Film
From Jan 1st, 1993 to Dec 31st, 1993	3.60	
From Jan 1st, 1994 to Dec 31st, 1994	0.00	

From Jan 1st, 1995 to Dec 31st, 1995	0.70	
From Jan 1st, 1996 to Dec 31st, 1996	1.15	
From Jan 1st, 1997 to Dec 31st, 1997	0.60	
From Jan 1st, 1998 to Dec 31st, 1998	1.40	
From Jan 1st, 1999 to Dec 31st, 1999	0.00	
From Jan 1st, 2000 to Dec 31st, 2000	0.20	
From Jan 1st, 2001 to Dec 31st, 2001	0.60	
From Jan 1st, 2002 to Dec 31st, 2002	1.10	
From Jan 1st, 2003 to Dec 31st, 2003	0.90	
From Jan 1st, 2004 to Dec 31st, 2004	0.20	
From Jan 1st, 2005 to Dec 31st, 2005	0.40	
From Jan 1st, 2006 to Dec 31st, 2006	0.50	
From Jan 1st, 2007 to Dec 31st, 2007	0.00	
From Jan 1st, 2008 to Dec 31st, 2008	0.00	
From Jan 1st, 2009 to Dec 31st, 2009	0.00	Dosimeter Film and Luminescent term
From Jan 1st, 2010 to Dec 31st, 2010	0.00	Luminescent term
From Jan 1st, 2011 to Dec 31st, 2011	0.00	
From Jan 1st, 2012 to Dec 31st, 2012	0.00	
From Jan 1st, 2013 to Dec 31st, 2013	0.00	
From Jan 1st, 2014 to Dec 31st, 2014	0.00	
From Jan 1st, 2015 to Dec 10th, 2015	0.00	
Total	**14.95**	

Obs.: This report presents the total effective dose received during the term of employment of an employee (Almeida D. M.)

4. CONCLUSIONS

A total of 12 phyla were identified growing on the liner of the FTC and the SFP as well as in and around the areas of the SFP drains of the Angra 1 NPP, between organisms not classified. The bacteria traveled the path from the edge of the SFP to the drains (outside of the pool), indicating that they have survived the exposure to radiation of 0.416 Gy/h (high radiation close to the racks).

The method of collection (SWAB) used in Angra 1 allowed the analysis of the biological material without any radiological risk.

Workers operating outside the SFP in Angra 1 and divers working inside the pool in many NPP were not exposure to high radioactivity.

5. REFERENCES

1. Almeida, D.M. 1999. Racks Supercompactos de Angra 1. *VII CGEN – Congresso Geral de Energia Nuclear, Belo Horizonte, Brazil.*

2. Almeida, D.M.. 2002 *Manutenção Subaquática do Sistema de Transferência de Combustível. XIII ENFIR* - National Meeting of Reactor Physics and Thermal Hydraulics *In: International Nuclear Atlantic Conference*, Rio de Janeiro, Brazil.
3. Almeida, D. M. 2016. *Bactérias no Canal de Transferência de Combustível e na Piscina de Combustível Usado da Usina Nuclear de Angra 1*.Master Dissertation Environmental Engineering Program. Rio de Janeiro Federal University. 75p.
4. Almeida, D.M.; Cabral, BC.A.; Dias, V.H.; Silva, R; Nassar, C.A.G. 2016. Bactérias sobrevivem na Piscina de Combustível Usado da Usina Nuclear de Angra 1 – Rio de Janeiro. *Proceeding VII Congresso Brasileiro de Gestão Ambiental, Campina Grande/-Brazil.* 1-6p.
5. Billi, D., Friedmann, E.I.; Hofer, K.G.; Caiola, M.G.; Ocampo, F. R. 2000 Ionizing-radiation resistance in the desiccation-tolerant cyanobacterium Chroococcidiopsis. *Applied Environmental Microbiology*, **66**, p.1489-1492.
6. Chicote, E.; Moreno, D.A.; Garcia, A.M.; Sarró, M.I.; Lorenzo, P.I.; Monteiro, F. 2004 Biofouling on the walls of a spent nuclear fuel pool with radioactive ultrapure water. *Biofouling*, **20**, p.35-42.
7. Eletronuclear – Eletrobrás Termonuclear S.A. Home page 2017. http://www.eletronuclear.gov.br.
8. IAEA. INTERNATIONAL ATOMIC ENERGY AGENCY. 1997.Further analysis of extended storage of spent fuel. In*: Final report of a co-ordinated research programme on the behaviour of spent fuel assemblies during extended storage (BEFAST-III) 1991-1996 (Viena).*
9. Leitão, A. C. 2017 *Efeitos somáticos das radiações ionizantes.* Capitulo IX: Radiobiologia e Fotobiologia.
10. Møller, A. P.; Mousseau, T. A.; 2013 The effects of natural variation in background radioactivity on humans, animals, and other organisms. *Biol. Rev. Camb .Philos. Soc.*, **88**, p.226–254.
11. Ragon, M.; Restoux, G.; Moreira, D.; Møller, A. P.; Garcia, P. L.; 2014 *Sunlight-exposed biofilm microbial communities are naturally resistant to Chernobyl Ionizing-radiation levels. Unite´ d'Ecologie, Systematique et Evolution* - CNRS UMR8079, Universite´ Paris-Sud, Orsay, France.
12. Rainey, F. A.; Ray, K.; Ferreira M.; Gatz, B. Z.; Nobre, M. F.; Bagaley, D.; Rash, B. A.; Park, M. J.; Earl, A. M.; Shank, N. C., Small, A. M.; Henk, M. C.; Battista, J. R.; Kampfer, P.; Costa, M. S.; 2005 Extensive diversity of ionizing-radiation-resistant bacteria recovered from Sonoran Desert soil and description of nine new species of the genus Deinococcus obtained *from a single soil sample.* Appl Environ Microbiol., **71**, n.9, p.5225-5235.
13. Ruiz G. M.; Czirják, G.; Genevaux, P. 2016 Resistance of feather-associated bacteria to intermediate levels of ionizing radiation near Chernobyl. Scientific Reports, **6**, p.229-269.
14. Sarró, M. I.; García, A. M.; Moreno, D. A. 2005 Biofilm formation in spent nuclear fuel pools and bioremediation. *International Microbiology,* **8**(3):223-230.
15. UCC – Underwater Construction Corporation. Home page 2017, https://uccdive.com/