The Effect of Gamma (γ) Irradiation to inactivate *Escherichia coli* in Contaminated water

H Ahari[1], M Alinejad Dizaj[2], S Paidari[3], A Anvar[4*]

[1]Assistant Professor, Department of Food Science and Technology Science and Research Branch, Islamic Azad University, Tehran, Iran

[2]Chronic Respiratory Diseases Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran.

[3]Department of Food Science. Technology Science and Research Branch, Islamic Azad University, Tehran, Iran

[4]Assistant Professor, Department of Hygiene, Science and Research Branch, Islamic Azad University, Tehran, Iran.

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Abstract

Current study was conducted to evaluate the efficacy of gamma irradiation to eradication of microbial contaminants from food industry water resources. Water samples were collected from food industries located around Tehran, capital of Iran. Samples from three major resources of food industry water system; Well, Tank and Recycled water were collected and one part of the samples irradiated with ^{137}Cs gamma radiator. Treated and untreated samples were tested by standard Multiple-tubes fermentation technique for detection of coliform bacteria and MPN indexes were calculated. Gamma irradiation had killing effect on coliforms and leads to noticeable reduction in MPN indexes of treated water samples compared to untreated ones. With few modifications in radiation dose, it is expected to implant portable gamma radiators to treatment of water in food industry section.

*__Correspondence__: [4]Assistant Professor, Department of Hygiene, Science and Research Branch, Islamic Azad University, Tehran, Iran. (e-mail: saaa4824@gmail.com).

Keywords: food industry, gamma-irradiation, water treatment, coliforms

Introduction

There has been a vast need for inactivation of different kinds of pathogens from drinking and waste waters. Traditionally, cholorization, ozonization and UV radiation processes have been used as the most common methods of disinfecting waters whilst Gamma radiation is being used more frequently due to concerns over by-products made by conventional methods such as cholorization and their contribution to increment of cancer risks and mortality. Between these pathogens (Thompson 2000) presence of Ecoli in water is conducted as the first source of water pollution.According to international standards Ecoliis transferred through oral and fecal contaminations and contaminated foods to human beings, in human body. It is presented as opportunist pathogen and cause urinal tract infections, septicemia, septic shock, new born meningitis as well as bird coli bacillus, pericardiagranoluma and salpenjite in birds and poultries(She, Shi, Xu, Zhou, Li, Tian, Li, Li, Dong & Ren 2016; Smith, Seymour, Moccia & Mothersill 2016; Zhang T. Y., Lin, Xu, Xia, Tian & Gao 2016a).

Gamma rays are high energy photons released from nucleus during radioactive delay process and has been used in a variety of situations for many years to inactivate micro-organisms e.g. medical products, food products and municipal sewages and sludge sewages. These days radiation method is used as portable method, which does not cause ionization of water and also any changes in physical and chemical properties of water. One of the main advantages of Gamma radiation consists of the fact that since irradiation is a physical process, no chemicals have to be added (Vilela, Oliveira, Vicentini, Casagrande, Verri, Cunha & Fonseca 2016; Zevallos-Concha, Nunez, Gasco, Vasquez, Quispe & Gonzales 2016; Zhu, Liu, Yu, Zhou & Yan 2016).

Microbial disinfection resulting from Gamma radiation is believed result from two general classes of reactions. The first direct ionization of Gamma photon energy and the second is, gamma radiation of aqueous material produces highly reactive unstable intermediates such as hydroxyl radicals, hydrogen atoms, and hydrated electrons. These highly reactive intermediates can cause chemical changes in the aqueous system and within microorganisms resulting in damage to the organisms in the system which, in turn, destroy particle associated micro-organisms(Hayati, Rezvani, Morsali & Retailleau 2017; Shirai, Miura, Yoshida, Yoshino, Ito, Yoshinari & Yajima 2016; Thang, Au, Rakovski & Prakash 2016).

Material and Methods

In this study, lactose broth, Brilliant Green Bile broth 2 %, EMB agar media have been used for cultivation of bacteria as well as Selective media (citrate, urea, MRVP, SIM, TST).Also Durham tubes were utilized to detect gas production.

Sampling

100 water samplesfrom poultry farm destinies wells,and tank water, were collected using sterile

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dishes (with 100 ml volume)near flame, from different a areas throughout the year, near flame samples were transferred to veterinary diagnosticlaboratory in less than 30 minute by help of ice. About 1/3 of samples were transferred to microbiology laboratory of veterinary college, Urumia university (tap and air pump were disinfected to avoid secondary pollution). Samples were divided into two groups, first group were directly transferred to microbiology laboratory for cultural process and were cultured on EMB medium.

Second group were transferred to nuclear physics laboratory and they were irradiated by cs(cs 137) with 20 milicurieactivity level for 6 hours The radiation device had 12 years half-life and was containedby lead walls all over radiation source.

After radiation samples were rapidly transferred to microbiology laboratory using ice to perform cultural and other test activities as mentioned for previous group. Then using 9 test tubes method they were tested.In this method at the first, lactose broth medium was made, first 3 test tubes contained strong medium (26 g/lit) and the other 6 remaining tubes contained weak medium (13 g/lit). All test tubes were contained small Durham tubes in a reverse form,therefore all tubes were contained 10 ml lactose broth.

10 ml water was added to the first 3 tubes 1 ml to the next 3 tubes and 0.1 ml water sample to the last 3 tubes. All tubes were incubated at 37 for 24 h. Tubes that did not contain gas in their Durham tubes were incubated for another 24 hours to obtain more assurance. if after 48 hours gas wasnot present it was countedas negative result otherwise, presence of gas in Durham tubes cause conducting of MPN testing for coli form counting. According to obtained result, 4 tubes werecontained gas bubbles which were separated from the other tubes and 10 ml 2%BGB broth was addedto them and 3-4 ml of sample from contaminated lactose broth tubes were added to the 2% BGB broth containing mj test tubes.

Presence of contamination caused gas production in Durham tubes. sample form contamination BGBbroth tubes were culture on EMB agarby slenle culture loop to observe colonies withmetallic greenish color if greenish color was not observe thenselective tests (TSI, SIM, MRVP),were conducted.

It is important to know that entrobacter kits are used instead of selective tests in these days. The other sample groups were under gone same process as mentioned above after radiation and transferring to the lab.

Results

According to obtained results, 3- δ radiated water samples out of all water samples, showed 42% contamination reduction. Radiationby 20 milicurie was effective on this pathogen (Tables 1-2).

Table 1 The number of colonies that obtained from the water that is not affected by Gama ray

		pipes with bubble with 10 cc of doubtful water	pipes with bubble with 1cc of doubtful water	pipes with bubble with 0.1 cc of doubtful water	mpn
A samples of waterof the watering Trough	Sample # 1	3	3	3	1100
	Sample # 2	3	3	1	460
	Sample # 3	3	3	2	1100
B samples of water of the source	Sample # 1	3	2	1	150
	Sample # 2	3	1	0	43
	Sample # 3	3	2	0	93
C samples of water of the village's well	Sample # 1	3	0	2	64
	Sample # 2	2	0	0	23
	Sample # 3	2	3	2	44

As Tables 1 shows, the maximum colonies that was found in watering Trough which had not been exposed to radiationwas 1100 colonies and the minimum was 460 colonies. The maximum number of colonies that was found in Source water, which had not been exposed to radiation was 150 colonies and the minimum was 43 colonies. Finally, maximum number of colonies detected in village's well was 64 whereas the minimum was 44 colonies.

Table 2 The number of colonies that obtained from the water that affected with the Gama ray

		pipes with bubble with 10 cc of doubtful water	pipes with bubble with 1cc of doubtful water	pipes with bubble with 0.1 cc of doubtful water	mpn
A samples of waterof the watering Trough	Sample # 1	3	3	1	460
	Sample # 2	3	2	0	63
	Sample # 3	3	3	1	460
B samples of water of the source	Sample # 1	3	1	0	53
	Sample # 2	2	0	0	23
	Sample # 3	3	1	0	43
C samples of water of the village's well	Sample # 1	2	0	2	20
	Sample # 2	1	0	1	7.2
	Sample # 3	1	2	1	15

As Table 2 exhibits, the maximum and minimum number of colonies has been changed dramatically due to the gamma radiation that has been radiated. The maximum number of colonies was 460 for watering Through and the minimum was 63.The maximum and minimum was 53 and 23 for the water of source. These numbers were 20 and 7.2 for villages well also.

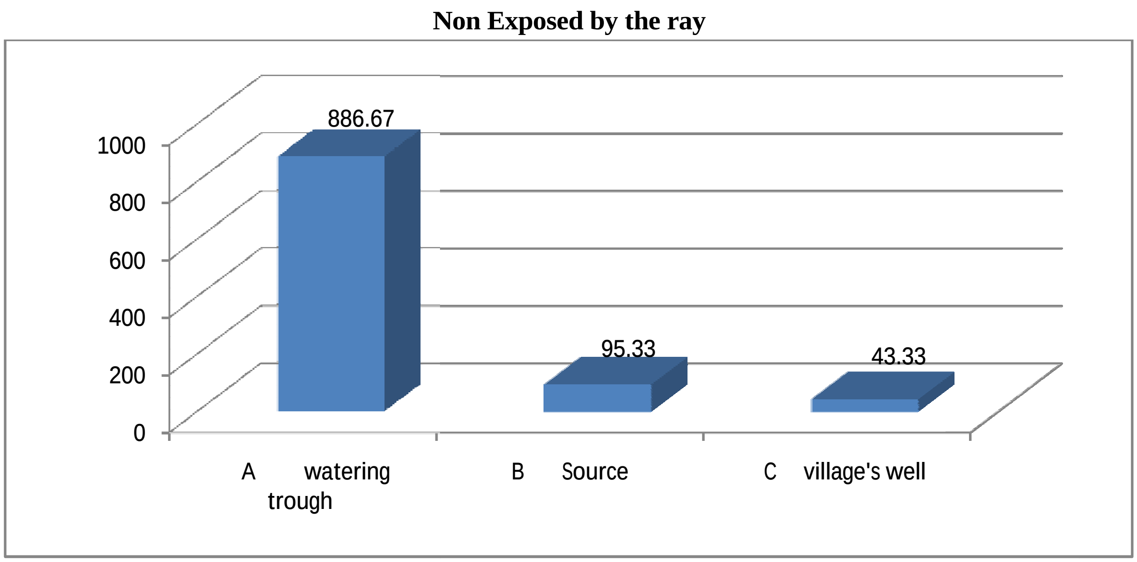

Fig. 1 Contamination level in water samples in non exposed by ray

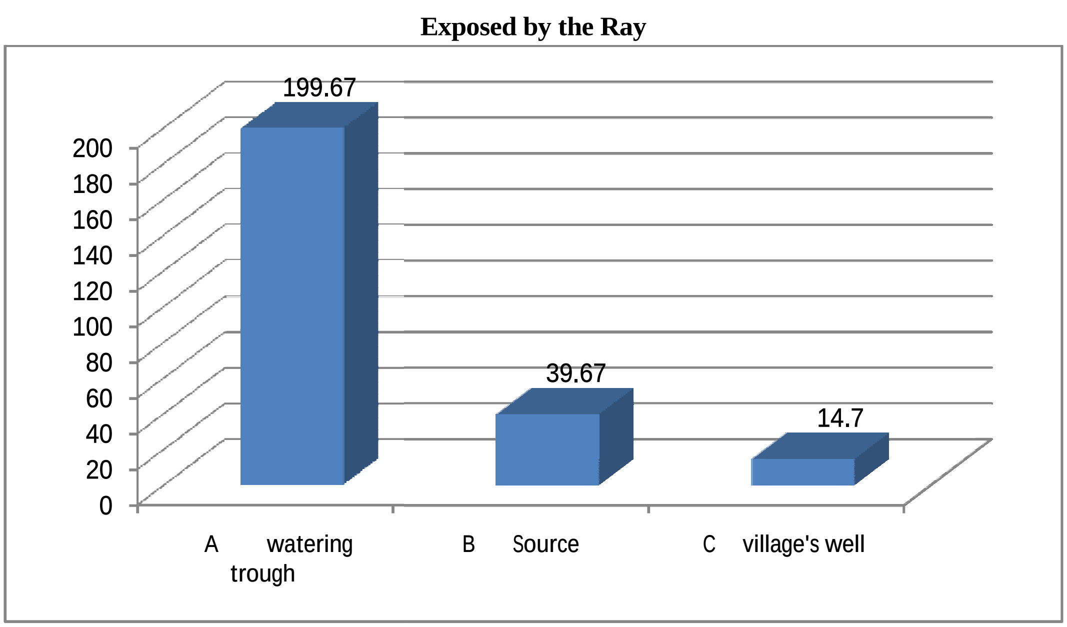

Fig. 2 Contamination level in water samples in exposed by ray

Discussion

δ Radiation is highly effective on water presented microorganisms specially ***E coli***, a summary of results are mentioned below. According to table 1, 2 maximum contamination levels was present in poultry farm destinies water samples, then in tank water and finally well water samples which had minimum contamination level.Contamination level in destiny water samples has reached from 886.67 to 199.67 (about 405 levels), in tank water from 45.33 to 39.67 and in well water sample from 43.33 to 14.7. Based on δ rediation level, bactericidal effects are different according to a study on 2016, 47% of stagnant water, *E. coli* were reduced using 28 milicurie dosage. Increasing dosage of radiation results in radiation time reduction(Smith *et al.* 2016).

Some researchers (Zhang Y., Yang, Zou, Chen, Ou, Zou & Wang 2016b) investigated the disinfection of waste waters by gamma radiation using 60co gamma source and high energy electrons. It finally observed 3 logs reduction of sample micro-organisms at an electron beam of 500 Krads, while at least 4 logs reduction were observed at the same time utilizing gamma radiation which, in turn, approve the obtained p value of $p< 0.03$.

Another study (Zevallos-Concha *et al.* 2016) was conducted to examine effect of gamma irradiation on coliphages, F-specific coliphages and Escherichia coli used as viral indicators, in urban wastewater treatment plant in Tunisia. They used ^{60}co as radioactive agent and kept the laboratory temperature between 25 to 30 ^{0}c. 1-9 KGy dose of radiation obtained by varying exposure time. Finally they concluded that radiation resistance depends on model micro-organism and matrix and Also E coli is deactivated much sooner than bacteriophages. The p value in this study was $p \leq 0.05$ and D10 for raw sewage was.06 +.005 and sewage and sludge was.07 ±.005.

Shirai *et al.* (2016) concluded that the characteristics of the water in which the microorganisms are suspended, a significant impact on their inactivation by means of radiation was observed. In a study made (Vereschako, Tshueshova, Gorokh, Kozlov & Naumov 2016) to inactivate using the Gamma radiation on micro-organism was investigated in which a Gamma cell 200 radiation chamber was utilized as well as a co-60 source. The average rate of irradiation was 1.4 KGy/s and a 170 Gy dose was used for 1-log inactivation of *E. coli* while, in another study founded by Zhang (2016), 60-80 Gy radiation for 1 log inactivation of *E. coli* in de-ionizing water was reported.

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Finally, Vereschako and et al proved that reduction in *E. coli* concentration has a linear relationship by the radiation dose(Vereschako *et al.* 2016).

Zhang Y. *et al.* (2016b) studied the effect of Gamma radiation of E-coli cultures combined with aeration. A bath reactor placed near a Gama cell 200 irradiator with co-60 source. The dose rate were in the range of 30-40 Gy/min applied to the sample and the concentration of final samples used of 10^7-10^8 E coli/ml. The Gamma radiation process was preceded by oxygen aeration and the inactivation efficacy of gamma irradiation was significantly improved by increasing the dissolved oxygen concentration in the suspending medium. Finally, they found, a dose of approximately 350Gy would appear to be sufficient to achieve four $\log_{10}$ units of pure culture *E. coli* inactivation in the media. Based on current investigation results, there is a possibility to use &radiation in stagnant water disinfection which are one of the most important factors in pathogen transfer to animals and humans.

In case of routine consideration intervals and usage of higher *δ*radiation it is possible to obtain ideal results without any chemical and physical changes in water as well as its ionization and in the minimum time to disinfect surface water.

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اثر اشعه گاما (γ) برای غیر فعال کردن باکتری اشرشیاکلی در آب آلوده

حامد اهری[1]، مریم علی نژاد دیزج [2]، سعید پایداری[3] و امیر علی انوار[4]*

1-استادیار، بخش مواد غذایی، واحد علوم تحقیقات، دانشگاه آزاد اسلامی، تهران، ایران

2-مرکز بیماری های مزمن تنفسی، موسسه ملی تحقیقات سل و بیماری های ریوی، دانشگاه علوم پزشکی شهید بهشتی، تهران، ایران

3- بخش مواد غذایی، واحد علوم تحقیقات، دانشگاه آزاد اسلامی، تهران، ایران

4- استادیار، بخش بهداشت مواد غذایی، واحد علوم تحقیقات، دانشگاه آزاد اسلامی، تهران، ایران

چکیده

چند روش مشترک برای ضد عفونی آب در این بخش استفاده می شود، اما با استفاده از روش (به عنوان مثال کلر) با خطرناک توسط محصولات همراه و همچنین به تغییرات قابل توجه در بو و طعم آب منجر شود. اشعه گاما به عنوان یک روش در حال ظهور است در حال حاضر توسط FAO / WHO برای آب، تصفیه خانه فاضلاب و مواد غذایی تشویق شده است. باکتری متعلق به آلاینده مشترک از آب و به عنوان یک شاخص اثر بخشی اقدامات ایمنی آب، از جمله اشعه گاما استفاده می شود. مطالعه حاضر با هدف بررسی اثر پرتو گاما به ریشه کن کردن آلودگی های میکروبی از منابع آب در صنایع غذایی انجام شده است. نمونه آب از صنایع غذایی واقع در اطراف تهران، پایتخت ایران جمع آوری شد. نمونه ها از سه منبع عمده ای از سیستم آب صنایع غذایی؛ خوب، مخزن و آب بازیافت جمع آوری شد و بخشی از نمونه تحت تابش با Cs137 گاما رادیاتور. درمان و نمونه درمان نشده با استفاده از روش تخمیر چند لوله استاندارد برای تشخیص باکتری کلی فرم ها و شاخص MPN مورد آزمایش قرار گرفتند، محاسبه شد. پرتو گاما اثر قتل بود در کلیفرمها و منجر به کاهش قابل توجه در شاخص MPN نمونه آب تصفیه شده در مقایسه با آنهایی که درمان نشده. با چند تغییر در دوز تابش، انتظار می رود برای کاشت رادیاتور گاما قابل حمل به درمان آب در بخش صنایع غذایی.

کلمات کلیدی: صنایع غذایی، گاما تابش، تصفیه آب، کلی فرم

*نویسنده مسئول: saaa4824@gmail.com

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