

# The Effect of the Anthropogenic Impact on Microbiocenosis of Caspian Sea

N. H. Rustamov, G. A. Guliyeva

National Academy of Sciences of Azerbaijan, Institute of Chemical Problems. Acad. M.F. Nagiyev Baku, Azerbaijan  
Republican Sanitary-Quarantine Inspection of the Ministry of Health. Azerbaijan, Baku, Azerbaijan

**Abstract** – The purposes of our researches were the determination of the level of purity of wastewater discharged into Caspian Sea, also the control over the content of cadmium and its extraction-photometric determination in fish and fish product. Samples of wastewater were taken on wastewater treatment plant in the Hovsan village, where are applied the complex of methods for purification of wastewater. Enterobacteriaceae causes especial interest in the case of anthropogenic pollution of Caspian Sea by wastewater. Salmonella, Proteus, Citrobacter - are risk factors for health of population. So, in analyzed material representatives of Enterobacteriaceae in samples of water were 18.3%±, in fish were 32.5%± from all isolated microflora. Klebsiella and Serratia were found in the studied objects as single strains, Vibrio parahaemolyticus were found in fish in the amount of 16.2%. We worked out the technique of two stage extraction-photometric determination of cadmium with 1,10-phenanthroline (phen) and alizarin yellow P (A and P). The technique allows to identify 0,5-20µg of cadmium in the volume 50 ml of aqueous phase with sufficient accuracy. Thus, the received data give the evidence of significant pathogenicity of dominant in our material *Vibrio parahaemolyticus*, which disseminates the fish and water in its habitats, and of epidemiological risk, which is created in environment by above-mentioned microorganism. Considering aforesaid, can be made a conclusion that it is required a constant control over the content of exactly cadmium in seafood, because, the excess of cadmium in human organism, led to substitution of zinc from many enzymes and can cause the cancer.

**Keywords** – Alizarin Yellow P, Caspian Sea, Detection, Enterobacteriaceae, Microflora, Photometric Determination of Cadmium, Wastewater.

## I. INTRODUCTION

In the conditions of natural and anthropogenic pollution, the methods of water environment control acquire a special importance due to their quickness, information content, availability and mass character. These methods are biotesting methods that allow evaluation of the biological danger of the water pollution by toxic substances [1,2].

Caspian Sea, which washes the coastline of Absheron peninsula, is the unique basin and plays an important role in the economy of countries, situated on its shore. In recent years Caspian Sea pollution, which is exacerbated by the development of marine oil-gas mining, and also by withdrawing of the effluent from oil, oil refinery, petrochemical, chemical, engineering and etc. industries, is becoming more serious problem [3,4].

The essential components of Caspian Sea pollution are heavy metals, especially cadmium [5].

Heavy metals pollutants, getting into the basins with wastewater of industrial companies, cause irreversible changes of natural ecosystems. It is known that cadmium is highly toxic cumulative poison, which affects kidneys and liver [6]. In oysters and liver of fishes, it can accumulate in significant quantity.

The main river of Azerbaijan, Kura, has the extremely important value in national economy, especially for irrigation, energetics, navigation, water supply, public health and fish industry [7,8].

Water and fish are the basis for the transmission of infective matter of microorganisms. Wherein the metabolism of microorganisms is changing, however, they do not lose their pathogenicity, getting into the habitual environment of warm-blooded [9].

According to statistics, fish and invertebrates cause bacterial infections, associated with water and food factor, in human. In the etiology of these diseases are *Salmonella* and other *Enterobacteriaceae*, which evidenced of the necessity of studying the content of bacterial microflora, that is dangerous for people, when it is located in fish and fish products [10]. The wide spread of microflora in water and fish, and the proved pathogenicity of many species for fishes and human, make it possible to use microflora as an indicator for objective evaluation of sanitary-hygienic condition in one ecosystem. Situation, emerged with microflora of water and fish, necessitate the revision of existing standards [11] determination of the level of purity of wastewater discharged into Caspian Sea, also the control over the content of cadmium and its extraction-photometric determination in fish and fish product.

## II. MATERIALS AND METHODS

The wastewater of Absheron peninsula was used as a material for study, on purpose to detect its contamination with bacterial flora.

Samples of wastewater were taken on wastewater treatment plant in the Hovsan village, where are applied the complex of methods for purification of wastewater. These methods include combination of mechanical treatment, neutralization of industrial waste, or reagent and biochemical treatment.

During the performance of the study were used commonly accepted in bacteriology methods of indication and identification of microorganisms [9].

The tested water samples were filtrated through 47 mm membrane filters, then, according to the guidelines for sanitary-microbiological analysis of the water of surface basins, 1 ml of each sample was inoculated in 9 ml of growth medium by the serial dilution method.

Express tests Compact-Dry were used in this work, that is considered as easy test method for counting of microorganisms.

The growth of colonies is appeared as a pigmentation of different colors, which emerges on chromogenic substrate, due to redox-indicators. With the help of Compact Dry trays, the membrane filters were also tested, by placing

filter on the nutrient tray, moistened with the saline. Filter has to be placed the collecting side up. For *Vibrio parahaemolyticus* were used Compact Dry vP. These microorganisms were extracted from fishes, living in the Caspian Sea, - carp, zander, silver carp, kutum and others (Figure 1).

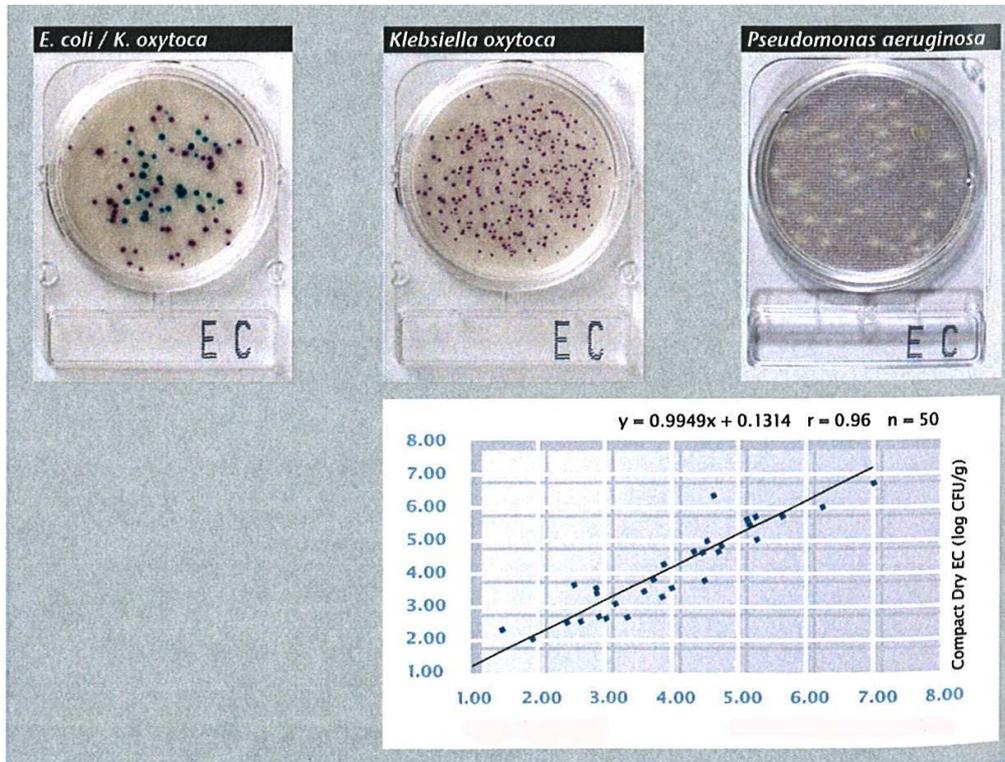


Fig.1. Detection of microorganism *Enterobacteriaceae* in sea water

In natural conditions, *Vibrio parahaemolyticus* lives in coastal waters, and in summer months its concentration increases. *Vibrio parahaemolyticus* is found in the marine environment, in seafood and in feces of the patients with the signs of acute enteritis. Compact Dry helps to identify

and differentiate *Vibrio parahaemolyticus* from other vibrio, due to the special chromogenic substrate that is specific for given microorganism, at which the growth of blue-green or blue colored colonies take place. (Figure 2)

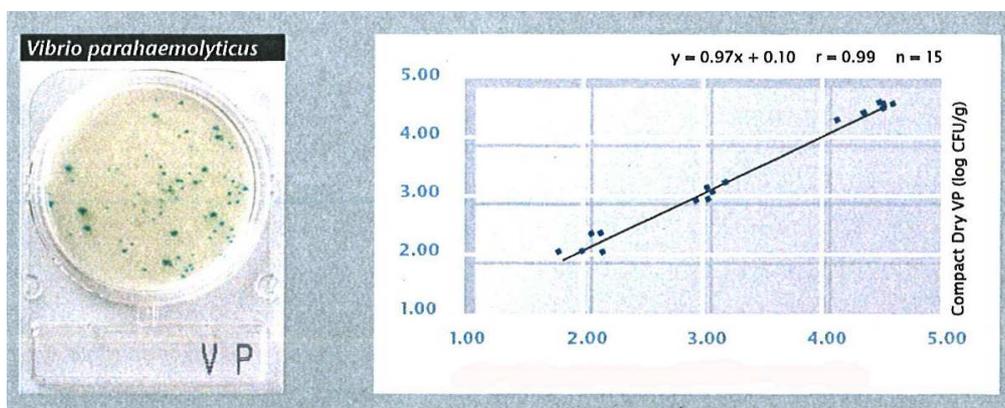


Fig. 2. Detection *Vibrio parahaemolyticus* in Caspian sea water and fish

Analytical control of the cadmium content has to be performed by sufficiently reliable method by Rustamov N.H., Guliyeva G.A. (2009) [12].

We worked out the technique of two stage extraction-

photometric determination of cadmium with 1,10-phenanthroline (phen) and alizarin yellow P (A and P). The technique allows to identify 0,5-20µg of cadmium in the volume 50 ml of aqueous phase with sufficient

accuracy. The essence of the two stage extraction-photometric determination of cadmium with phen, A and P is: on the first stage cadmium is separated from associated elements, as the solvated iodide compound, extracted by solution of tributylphosphate in benzene; on the second stage cadmium is determined with phen, A and P in organic phase. The technique was applied for the determination of cadmium in several samples of fishes.

The weighed 25 g of fish, taken in porcelain crucible with known constant weight, was dried in drying cabinet for removal of the moisture. Further, it was turned into ash with slow increasing of temperature in the covered crucible. The crucible was placed on a tripod high enough above the flame of burner, and then the heating was increased. After the evaporation and cessation of gas evolution, the residue was treated by several drops of HNO<sub>3</sub> (1:1) and turned to ash at the temperature 250-300 °C. Then the residue again was treated by several drops of HNO<sub>3</sub> and this time was turned to ash at temperature 450-500 °C until the receiving of white precipitate. In cooled ash was treated by 5 ml 2 NH<sub>4</sub>Cl, and crucible was placed in a boiling water bath, the solution was mixed thoroughly by glass stick and then filtrated into 50 ml beaker. Solvating of the residue was repeated again with 5 ml 2 NH<sub>4</sub>Cl, and then with 5 ml of distilled water. pH of united solution was adjusted to 3-4, that was controlled by ionomer 713-74.

For the determination of cadmium with phen, A and P the solution was placed into the separating funnel, 0.5 ml 1 M KJ, 5 ml of acetate buffer with pH 7.0 were added to it. The volume of aqueous phase was brought up to 25 ml with distilled water. To the solution was added 5 ml of the mixture of benzene with TBP (1:4) and then it was shaken for one minute. After separation of the layers 3 ml of organic phase were removed into the tube with a glass stopper, then 0.3 ml 1×10<sup>-2</sup>μ of phen, 0.3 ml 5×10 μ A and P and 2 ml of buffer solution with pH 7.0 were added to it. The volume of aqueous phase was brought to 10 ml and further 2 ml of benzene was added. After shaking for one minute and separation of the layers, the optical density of the complex extract was measured relatively to reagent extract.

### III. RESULTS AND DISCUSSION

*Enterobacteriaceae* causes especial interest in the case of anthropogenic pollution of Caspian Sea by wastewater. *Salmonella*, *Proteus*, *Citrobacter* - are risk factors for health of population.

So, in analyzed material representatives of *Enterobacteriaceae* in samples of water were 18.3%±, in fish were 32.5%± from all isolated microflora.

*Klebsiella* and *Serratia* were found in the studied objects as single strains, *Vibrio parahaemolyticus* were found in fish in the amount of 16.2%.

Also were isolated the water strains of *Ps. aeruginosa* that have the ability to survive in wastewater during different types of treatment. Aeromonads were found predominantly in marine water in amount on the average equal to 12.3%. The results of the seasonal dynamics of

microorganisms' pathogenicity witnessed the increase of fish strains in the Caspian Sea and Kura from spring to autumn. Herewith, the hydromicroflora in the sea and river practically had not change the indices of pathogenicity that provides the stability of bacteriocenosis.

The content of cadmium in fish was defined according to calibration graph.

Table 1. The content of Cd in fishes (mg/kg)

Sample Number	The Determined Content of Cd (mg/kg)
I sample (carp)	0.243 ± 0.03
II sample (silver carp)	0.276 ± 0.03
III sample (zander)	0.284± 0.03

In tested samples, the content of cadmium was also determined by atomic absorption method on AAS 300, Perkin Elmer, USA 2000. According to the obtained results, the content of Cd in I sample was 0.25 mg/kg, in II sample -0.27 mg/kg, in III sample -0.29 mg/kg, respectively; whereas, the normal content of cadmium in the fresh fish is 0.2 mg/kg.

### IV. CONCLUSION

Thus, the received data give the evidence of significant pathogenicity of dominant in our material *Vibrio parahaemolyticus*, which disseminates the fish and water in its habitats, and of epidemiological risk, which is created in environment by above-mentioned microorganism.

Considering aforesaid, can be made a conclusion that it is required a constant control over the content of exactly cadmium in seafood, because, the excess of cadmium in human organism, led to substitution of zinc from many enzymes and can cause the cancer.

### REFERENCE

- [1] Obuhova O.V., The influence of salts of heavy metals on growth and factors of pathogenicity of opportunistic bacteria // Hygiene and sanitary, 2011, N.1, pp. 37-39
- [2] Krasovskiy G.N., Yegorova H.A., Bichkov I.I. Classification of dangerous of substances polluting the water // hygiene and sanitation, 2006, N2, pp.5-9
- [3] Sharifzadeh G., Guliyeva G., Khalilov R., Detection of cadmium in the foodstuffs and its toxic influence of them to human organism / Natural cataclysms an global problems pf the modern civilization. Baku-Innsburk, 2009, pp. 53-58
- [4] Katunin D.N., Kurochkina T.F., Popova O.V. et al. Fisheries research in the Caspian Sea. Results of SRÜ caspy for 2001. Astrakhan, 2002, pp.37-41
- [5] Arvand M., Pourhabib A., Afshari A. et al. Determination of cadmium and zinc in water samples by flame atomic absorption spectrometry // Journal of analytical chemistry, 2008, vol. 63, #10, pp.1050-1055
- [6] Science without Borders. Transactions of the International Academy of science // H.E., 2007/2008, v.3
- [7] Methodological guidance (4.2.1884-04) of sanitary-microbiological analysis of water of surface basinsm. 2004
- [8] Influence of anthropogenic impacts on the aquatic environme

nt and microbiocenosis cage fish hatcheries / Yuhimenko L. N., Litov A.V., Pime-nov A. V <http://www.pisciculture.ru/articles?id=41>. HTML Портал @pisciculture.ru” / 2007

- [9] Obuhova O.V., Larceva L.V., Lisickaya I.A., Sanitary-microbiological evaluation of hydroecosystem of Volga delta at anthropogenic pollution // Hygiene and sanitary, 2009, №1, pp. 23-25
- [10] Daraseliya G.Y., Biological safety of foodstuffs: Textbook, Astrakhan, 2006
- [11] State standards. Raw materials and food products. Methods for determination of toxic elements. M.: ИПК standards publishing House, 1996
- [12] Rustamov N.H., Guliyeva G.A. Extraction-photometric determination of cadmium in meat // Abstracts of VII All-Russian Conference on analysis of objects of ecoanalytic's environment, Yoshkar-Ola, 2009, pp.186-187

## AUTHOR'S PROFILE



Gulnara Amir Kuli Guliyeva was born in 1958 in Baku, Azerbaijan Republic. In 1983 graduated Azerbaijan State University with a degree in Microbiology. In 1989 she worked as a bacteriologist in the Laboratory of Especially Dangerous Infections at the Republican center of Epidemiology of the Ministry of Health of Azerbaijan. Since 1997 till now she is working in

Republican Sanitary-Quarantine Inspection as microbiologist and is a head of microbiological laboratory.

In 2006-2009 she performed the collaboration scientific researches in Institute of Chemical Problems of Academy of Science of Azerbaijan under the supervision of Rustamov R. X.

In 2013 defended the thesis for the degree of PhD in Microbiology under the supervision of honored scientist prof. Sadikhova F.E.

Since 2015 she is a teacher of the Department of Ecology on the course of the subject “Safety of the vital functions” in Western University in Baku.

Has numerous scientific papers and publications, also participated at different conferences in Turkey, Germany, Italy and Belgium.

Most important works of author:

- 1.Sadikhova F.E., Guliyev G.A., Ahundova N.Sh., Mamedova K.G. Results of microbiological control of imported food with regard oppurtunistic fungi of the genus aspergillus and penicillium // The progressiv researches «Science and genesis», 2015, N1, pp. 46-49
2. Sadikhova F.E., Guliyev G.A. Antiviral and antibacterial screening of chemical compounds, nicotinic and derivatives // Global science and innovation, 2015, june 24-25, pp.204-207
3. Guliyev G.A., Yakovleva O.V., Babayeva E.M. Sanitary-bacteriological characteristics of drinking am waste water Absheron district Sulu-Tepe and Bird array / Materialy XI Międzynarodowej naukowo-praktycznej konferencji “Wykształcenie i nauka bez granic”, 2015, vol. 12, pp.39-46