Microbiological monitoring of water quality used for water supply and assessment of water sources toxicity (the case of Elista city, the Republic of Kalmykia)

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Abstract. Water quality indicators taken from different sources over the Republic of Kalmykia vary significantly both by year and season. Therefore, an urgent need arose to develop a system targeted at regional and local monitoring of water resources. The system will involve modern monitoring technologies in real time and remote sensing techniques. In the Republic arsenal, there are some required practices. Electronic maps (for monitoring water resources) were developed at scale of 1:200000; databases are being under development. Improvement and development of the Republic’s water utilization system should be based on the principles of ecosystem water use, providing for strict monitoring and control over quantitative and qualitative indicators of water resources based on a technologically advanced monitoring system including the options regulating standardization, eco-quality, and economical consumption.

1. Introduction
The aim of the study is to analyse the main problems caused by water supply in the Republic of Kalmykia. Study objectives are to: a) conduct a microbiological monitoring of water quality in places of water intake used for water supply of Elista city; b) assess the efficiency of water toxicity (with the existing methods); c) assess the efficiency on how water self-purification systems work (water intake sources are in Elista city); d) verify the laboratory and analytical studies of water samples taken from the urban water intake; e) conduct a study evaluating the efficiency of water purification according to generally accepted methods used to do the quality test for drinking water.

To achieve the aim and objectives, we carried out some laboratory and analytical studies with water samples taken from the urban water intake. Based on the results, we assessed the efficiency of water purification along with the conventional methods in use.

2. Methods and materials
Laboratory and analytical studies of water samples taken from the urban water intake and in the process of water purification

Laboratory and analytical studies of water samples taken from the urban water intake and in the process of groundwater purification are carried out according to some special and general quality indicators.

Common indicators include:
• mineralization (M);
• total stiffness (Ж);
• }
permanganate oxidation (O);
• temperature (T);
• acidity pH;
• chlorides (Cl−);
• sulfates (SO42−);
• nitrates (NO3−);
• fluorine (F−);
• iron (Fe);
• manganese (Mn);
• copper (Cu2+);
• zinc (Zn2+);
• lead (Pb2+);
• petroleum products (n / n).

The special indicators include a set of determinations performed in a full chemical analysis; the content of phenol, organochlorine pesticides, benzopyrene, specific substances to specific objects, surfactants (SAS).

When analysing the water quality, it is mandatory to count microbiological and organoleptic indicators.

Identification of the general indicators is carried out in accordance with some regulatory documents:
• dry residue – GOST 18164-72;
• total stiffness – GOST 4151-72;
• chlorides – GOST 4245-72;
• sulfates – GOST 4389-72;
• nitrates – GOST 18826-73;
• fluorine – GOST 4386-81;
• iron – GOST 4011-72;
• zinc and lead – GOST 18293-72;
• copper – GOST 4388-72;
• manganese – GOST 4974-72.

Permissible norms (quality indicators) that determine the possibility to use underground waters for drinking purposes are characterized by the special requirements. As known, the quality of drinking water is determined by the requirements of GOST 2874-82 "Drinking water". For drinking water, permissible norms/standards include the analysis of the maximum permissible concentration (MPC) in underground waters of certain chemicals, the maximum permissible values (MPD) of such microbiological indicators as the coli index and coli titer, and MPD of organoleptic indicators.

Indicators of background and maximum permissible quality show, respectively, \( C_\phi \) and \( C_n \), where, as a rule, \( C_\phi < C_n \).

There are two degrees of pollution:
1 – is prelimit, when a pollutant substance concentration is more than \( C_\phi \), but less than \( C_n \), i.e. \( C_\phi < C < C_n \) or \( \bar{C} < \bar{C} < \bar{C}. \)
where $\bar{C}_n = \frac{C_{\phi}}{C_n}$, $\bar{C} = \frac{C}{C}$.

$II$ – the prohibitive concentration of a pollutant is greater than $C_n$, i.e. $C > C_n$ or $C > I$.

The first degree is the initial stage of pollution. In the 2nd degree, the following sub-degrees (gradations) are pointed out:

$IIa. I << 10; IIb. 10 << 100; IIc. > 100$.

Graduation $IIb$ characterizes the extreme pollution degree.

Types of underground water pollution.

The main types of underground water pollution are chemical and thermal. Chemical pollution is very common. Often chemical and thermal pollution appear together.

Chemical pollution is divided into the following types (characterized by a water quality indicator or the content of the predominant polluting component in it): mineralization, general hardness, chloride, nitrate, sulfate, fluorine, heavy metal (copper, zinc, lead), hydrocarbon, pesticides (mainly, organochlorine), general organic (characterized by oxidizability permanganate).

The main feature of thermal pollution is increased water temperature compared to the background water temperature.

The listed types of chemical and thermal pollution should be reflected when the main sources of underground water pollution are studied.

The water pollution index (WPI) and the limit of permissible concentrations (MPC) in the places of water intakes in Kalmykia, including the urban water resources in Elista city are given in table 2.5.

| Name                              | Pollution index | Water pollution class | MPC                          |
|-----------------------------------|-----------------|-----------------------|------------------------------|
| River Kuma                        | 4.86–5.62       | 3                     | sulfates – 3.65, copper – 5.95, petroleum products – 1.36, aluminum – 7.2 |
| Kumo0Manychensky canal            | 2.7             | 4                     | molybdenum – 2.2, iron – 2.0, copper – 1.8 |
| Tersko-Kumsky Canal               | 1.5             | 2                     | salts – 1.4, sulfates – 4.54, zinc – 1.47, copper – 7.3 |
| Chernozemelsky canal              | 2.99            | 4                     |                                |
| River Vostochny Manych            | 3.57            | 3                     | salts by dry residue – 2.37, sulfates – 8.49, chlorides – 2.16, iron – 1.8, manganese – 1.51, petroleum products – 1.9, magnesium – 2.8 |
| Chograisk wayer reservoir         | 2.62            | 4                     | -                            |
| Lake Manych-Gudilo                | 68.2            | 5                     | -                            |

3. Results

Studies analysing the efficiency of water purification according to the generally accepted methods

The study aimed to assess the efficiency of water purification according to the generally accepted methods.

Assessment of the water samples quality taken from the distribution water network of Elista city that was conducted according to organoleptic and physico-chemical indicators based on GOST and unified research methods, showed:

- the analyzed water samples are characterized by unsatisfactory organoleptic properties (exceeding the recommended regulations for colour and turbidity);
- according to the salt composition, the studied water sample belongs to the hydrocarbonate class of calcium type water and meets the requirements of GOST 2874-82 “Drinking water” and modern hygienic requirements set to the quality of drinking water, while the calcium content is 0.65mg/l, the
content of the main elements in fractions from the MPC are: calcium – 0.46, magnesium – 0.27, bicarbonates – 0.56, chlorides – 0.52, sulfates – 0.22 units;

- the study of the content of inorganic elements, normalized by the sanitary-toxicological criterion of harmfulness of the 1st and 2nd hazard classes (heavy metals, non-metallic elements), showed that in the test water sample was determined: sodium at a concentration of 65mg/l (0.32 MPC), barium – 0.13mg/l (1.3 MPC), strontium – 1.1mg/l (0.16 MPC), arsenic – 0.002mg/l (0.2 MPC), biologically active element boron – 0.39mg/l (0.8 MPC), a comprehensive indicator of the content of inorganic elements of the 1st and 2nd hazard classes exceeded the recommended value (less than 1) by 2.8 times;

- of metals normalized along with the organoleptic criterion of harmfulness, iron was determined mainly at the concentration of 0.53mg/l, exceeding the hygiene regulations (less than 0.3mg/l) by 1.7 times;

- the content of organic pollutants (nitrogen-containing compounds, trihalomethanes, organochlorine pesticides, polyaromatic hydrocarbons, anionic surfactants) revealed the excess of hygienic regulations for trihalomethanes. The complex indicator in trihalomethanes group, which are formed during chlorination of water, revealed its excess by 3.7 times, mainly due to the high content of chloroform and dibromochloromethane;

- the biologically necessary fluorine element in the test sample was determined at the concentration of 0.13mg/l, which is significantly lower than the recommended standard for the fourth climatic zone (0.6 mg/l).

According to the corrosion activity indices (P1 and P2), the studied water sample corresponded to the recommended standards.

An integrated assessment of the chemical composition done towards the drinking water samples, which are under the bio-test conducted with hydrobionts, showed that the water sample is toxic for daphnia and correlates with the results showing trichloromethanes content.

The most widely used and generally accepted method for assessing the water quality is sampling taken from the points of use (POUs) by the traditional methods based on using some bacterial cultures. This method of sampling allows to determine the quality of the water supply system as a whole, but can be carried out at each point only once per two weeks. Such a rare sampling, combined with a retrospective of the results obtained, makes it difficult to effectively and timely assess some risks and control the process. Besides, there is a risk to get a false-positive result due to the contamination of the samples during the sampling process or a false-negative result due to a low sensitivity of the method itself. Moreover, the methods’ efficiency, where the methods are based on the analysis of microorganisms growth, strongly depends on the chosen environment and incubation parameters. The emergence of a new technology that provides real-time analysis of the bio-burden of the water supply system will eliminate these limitations, as well as provide the effective risk reduction and process control.

A real-time bio-burden analysis system for water was carried out. In order to improve the equipment used to assess water quality, the Working Group [10] on the development of a real-time bio-burden analysis system for water (OWBA – Online Water Bioburden Analyzer) determined the user requirements and the test protocol for such a system, and also conducted assessment of the commercial advantages of its development [10–14].

The main objective of the group [10] is to help equipment suppliers create an OWBA system that meets the requirements of users and regulatory authorities. According to the members of the group, “the development and implementation of a real-time bio-burden water analysis system will help reduce the number of errors in sampling selection and testing by reducing the number of manipulations, as well as increasing the level of product safety and process control, due to the statistically significant data” [13].

According to the representatives of the group, the OWBA system is not a substitute for pharmacopoeial methods of water assessment, but serves as an additional tool to reduce some risks. They include reducing energy consumption and necessary labor resources, and also contribute to improving product quality and a better understanding of the process [12].
The Working Group [10] compiled the technical requirements for the system regarding bio-load sensitivity, calibration, chemical compatibility, operational parameters and the necessary consumables [13]. In addition, a detection limit was established (LOD – Limit of Detection), equivalent to the detection limit of culture methods (10 CFU/100ml). The analysis modes were identified that included continuous sampling, time-oriented sampling and daily routine operations. In general, according to the compiled requirements, the system had to provide continuous and periodic monitoring of critical control points (CCP – Critical Control Points) and POUs, and also have a sufficient level of sensitivity to detect microorganisms in water, including potentially harmful substances, such as iron oxide, residual disinfectants and sealing materials.

### Table 2. Commercial Benefits described in "OWBA Business Benefits Assessment" (document)

| Commercial benefits of the online water bio-burden analyzer |
|-----------------------------------------------------------|
| Energy saving                                             |
| Reduce cleaning cycles by evaluating system capabilities   |
| Lowering the operating temperature of the hot water supply system |
| Labour cost reduction                                     |
| Reduced sampling and laboratory testing due to online monitoring and optimization of the water supply system |
| Improving product quality and process understanding       |
| Reduce the number of investigations through continuous monitoring the water quality. |
| More rapid response to deviations of the microbiological composition of water |
| Improved understanding of the process and product safety through online monitoring. |
| Real-time production of intermediate products, buffers/solutions and the constituent water |

One of the technologies that meet the established requirements for OWBA is laser-induced fluorescence (LIF), which is a spectroscopic analysis method that is highly sensitive to fluorescent compounds. Fluorescence is the luminescent glow that occurs when a compound absorbs radiation of one wavelength and emits radiation with a different wavelength. Fluorescent compounds are also called fluorophores. The most widely known fluorophore is quinine, which is in tonic drinks.

LIF technology is already used to detect microorganisms in the methods of flow cytometry, capillary electrophoresis, solid phase cytometry, bioluminescence of adenosine triphosphate, and also associated with an increase in autofluorescence. However, a number of these methods involve staining the microorganisms to increase the radiation intensity. At the same time, the analysis of the internal fluorescence of the microorganisms eliminates the use of dyes and sample preparation, but requires highly sensitive equipment. The advent of more powerful lasers with different wavelengths opens up some new possibilities for LIF technology that can be applied for the analysis of weak fluorescence inherent in microorganisms.

The source of excitation for fluorescence is a laser-like light source. A laser with a suitable wavelength and intensity can cause fluorescence in microorganisms due to the fluorophores in them, such as tryptophan (Nicotinamide Adenine Dinucleotides – nicotinamide adenine dinucleotides) and flavins [17]. The wavelength of the excitation source depends on the emission spectrum of the analyzed fluorophones and should cause sufficiently strong fluorescence to detect as many biological materials as possible. Non-biological materials (plastic, rubber, paper) can also fluoresce, therefore, a special software is used to separate the fluorescence of such materials and microorganisms.

OWBA: real-time microbiological water analysis technology shows that with theLIF-based OWBA system, a microbiological water analysis can be performed in real time without the use of consumables and the limitations inherent in standard methods. Microbiological water analysis systems available on the market are equipped with a 405 nm laser. Such a laser is capable of simultaneously causing Mie scattering (Mie – Minimum Ignition Energy – minimum flash energy) and internal fluorescence of particles contained in a sample when it passes through the system, and interacts with an excitation source. Detection and analysis of Mie scattering and fluorescence provide real-time information about the presence and biological nature of particles. The method under
consideration is free from the drawbacks that the traditional methods have (based on a microbiological growth analysis) since its result does not depend on the correct choice of environment and incubation parameters.

In LIF-based systems, the internal fluorescence of particles is recorded with a photomultiplier tube (PMT – Photomultiplier Tube) – a sensor that is highly sensitive to light. The system can be equipped with one or two PMT. Systems with two PMT provide more efficient separation of non-biological materials, such as iron oxide, which meets the requirements for OWBA [13]. Each material and microorganism has a unique spectrum of excitation and radiation. The same wavelength of the exciting radiation causes wide spectrum fluorescence in some materials, and a narrow one in others. The coincidence of the emission spectra of biological and non-biological materials can also be used.

Figure 1 shows the emission spectra of various biological and non-biological materials at a wavelength of exciting radiation of 405 nm.

![Figure 1. The emission spectrum of two microorganisms and eight materials at a wavelength of exciting radiation of 405 nm. The graph shows the Raman scattering band, as well as two detection areas for a real-time microbiological analysis system with two PMTs](image)

In the figure, on both sides of the Raman band, the detection regions for PMT are marked. The Raman band means fluorescence resulting from the interaction of laser radiation with water. Therefore, to detect the particles in water, the detection region of the system should not coincide with this band. Under exciting radiation with a wavelength of 405nm, the Raman band for water reached the maximum value, approximately 469nm [18].

With two areas of PMT detection, the differences between the emission spectra of non-biological and biological particles can be used to determine inert biological material. Advanced data processing algorithms can be used to separate biological particles and interference material.

Online bio-burden monitoring, risk reduction and process control show that the real-time microbiological water assessment system allows conducting a continuous bio-burden monitoring and thereby increasing the actions' efficiency directed to risk reduction and process control. Comparison of the number of biological particles, determined with the IMD-W system that has the number of colony forming units, determined by the traditional method through the cups with TSA (TSA – Tryptic Soy Agar).

The data presented were obtained by the standard cup-based method and with the IMD-W™ system, developed on the basis of OWBA requirements. They cover a wide dynamic spectrum, and also testify to the high sensitivity of such systems and their efficiency in monitoring bio-burden.
Data, continuously obtained due to such systems, can be combined into a single database and used for analysis. An analysis of trends based on such data is much more effective than analyzing the results of episodic sampling obtained by the traditional methods. According to the USA law, water supply system is recommended being monitored at intervals “sufficient to confirm the controllability of the system and to obtain water of adequate quality” [15]. The most preferable approach is a continuous monitoring of equipment, which allows creating an extensive database of in-house data.

4. Conclusion
The study succeeded in solving the following tasks stated as objectives at the beginning:
1. the microbiological monitoring of the water quality has been conducted (the water was taken from the water intake sources used for water supply in Elista city); the efficiency of water toxicity (with the existing methods) has been assessed.
2. The efficiency on how water self-purification systems work has been evaluated (water intake sources are in Elista city);
3. To achieve the aim and objectives, some laboratory and analytical studies with water samples have been carried out (the water samples were taken from the urban water intake); on the results obtained the assessment towards the efficiency in water purification along with the conventional methods has been done.

Based on the results of a continuous monitoring, an extensive database can be created to analyze trends and assess the bio-burden level of the water supply system. An effective trend analysis will allow responding to deviations more quickly than it could be done with the traditional monitoring methods. Ultimately, the use of LIF-based systems will help improve product quality, improve process understanding, save energy and reduce the risks. To solve the problem of water supply in Elista city (the Republic of Kalmykia) with quality water, LIF-based systems for real-time monitoring of bio-burden is recommended to use.

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