SPECIFIC USE OF *PHRAGMITES AUSTRALIS* FOR RADIATION MONITORING*

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Abstract. *We compared the methods of sampling and analysis of radionuclide concentrations in samples of common reed in the Chornobyl Exclusion Zone. Selection and analysis of samples were generated using two methods. The first one is consistent with the current regulations, which allow selection and analysis of the whole plant of common reed and calculations of radionuclide concentrations in relation to the wet sample's weight. The second method, adopted in applied ecology, directs the selection of individual plant organs and the calculation of radionuclide concentrations in relation to the weight of the dry sample. It has been established experimentally that when applying the second observation method, the statistical uncertainty of measurement is 2.5 times smaller, which makes this method more suitable for radiological control and monitoring.*

Key words: *Chornobyl exclusion zone, field radiation measurements, radioecological monitoring, uncertainty of measurement results, Phragmites australis.*

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1. Introduction

For biological indication during radio-ecological control and monitoring of the environment, one of the most important metrological characteristics, which determine the reliability and reproducibility of observation results, is the uncertainty of measurement. [5]. This parameter most accurately characterizes the quality of the measurements of radionuclides in living organisms or their parts, which are used as biological indicators of the environment. Uncertainty of measurement, in this case, depends on sampling errors, laboratory measurements, allocation of radionuclides in biological indicators, and affects the number of dimensions in the implementation of the environmental mapping [1]. During the study period, which followed the 1986 disaster at the Chornobyl Nuclear Power Plant (Ukraine), the primary attention regarding the accuracy of measurements was paid to laboratory analysis of radionuclides. The dependence of accuracy of radio-ecological observations on features of field sampling has been developed less. Researchers pay particular attention to allocation of radionuclides in the soil and the influence of this factor on the methodological features of soil sampling [3]. In radio-ecological studies of vegetation more attention was paid to methodological features of leaf selection, pine needles, wood and bark of woody vegetation. Methodological features of selection of herbaceous vegetation received less attention. At the same time, operating procedures govern the location and frequency of sampling, but do not describe the requirements for the parameters of the samples taken [8]. In the practice CEZ study of aquatic vegetation are based on traditions of national radioecology, which Instructs the selection and analysis of the whole plant and the calculation of radionuclide concentrations considering the green weight of the sample [4]. This approach is also applied to the CEZ with regard to common reed, the samples of which are taken and analyzed in the form of a non-dissected plant with fragments of plant roots and rhizomes. Besides, our studies of radionuclide accumulation by individual tissues and organs of plants [2] showed the effect of this methodological approach on the quality of measurements in environmental monitoring. Since the value of common reed for biological indication for monitoring of aquatic ecosystems in the CEZ has grown in recent years, it becomes more topical to investigate this plant as a biological indicator [9].

The aim of our study is to analyze the components of the total statistical uncertainty of measurement associated with sampling measurements of ^{90}Sr and ^{137}Cs accumulation of common reed.

2. Material and methods

The observation was conducted in 2008-2013 in ecosystems of six reservoirs (surveillance places) in CEZ: Lake Azbuchyn, Lake Glyboke, Lake Daleke, Yaniv backwater of the Prypiat River near the Prypiat town, the cooling pond of the Chornobyl Nuclear Power Plant (NPP pool), alignment of the Prypiat River near Chornobyl (Chornobyl transit). During observations, *Phragmites australis* (Cav.) Trin. ex Steud were sampled. Sampling and analysis has been implemented using two methods. The first one has been done according to the current instructions in CEZ, allowing the selection and analysis of the whole plant of

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common reed and calculating the concentration of radionuclides regarding the green weight of the sample [8]. For the first method, are one or two whole plants were collected monthly during growing season.

The second method, which we used for comparison, involves the selection of certain plant organs and calculating concentrations in relation to the weight of the dry sample. Leaves and stems of common reed fragments were collected at mid-height of the plant. For comparison, in some cases, upper and lower leaves of the plant were selected too. Sometimes, the plant roots were studied as well. For this method, samples of respective parts of 30 plants were collected, monthly during the growing season.

From leaves and stems of common reed freshly drawn using the second method of raw samples, according to current regulations [7], aqueous extracts were prepared. Extraction was carried out for 12 hours at a ratio of weight (grams) sample / water - 1/10. The aqueous extract was filtered and sent to the radio-spectrometric and electrochemical analysis.

Electrochemical analysis of the content of Ca^{2+} ions and K^{+} in aqueous extracts of leaves was carried out using the appropriate ion-selective electrodes and ion meter pX-150MI. Ion concentration was calculated in relation to the dry weight of samples.

In the laboratory of SSE "Chornobylsky special plant" the analysis of the specific activity of ^{137}Cs and ^{90}Sr in dry samples of selected whole plants and in aqueous extracts of common reed leaves. The analysis was performed using a spectrometer of beta energy radiation SEB 01-150 and gamma spectrometer with an analyzer 4900 Nokia LP B and Ge-detector.

The total uncertainty of measurement (u) was calculated in accordance with the recommendations [5] by the formula:

$$u(\%) = \sum_c u_c(\%) , \qquad (1)$$

where: u_c is composing uncertainty of measurement. In this case, we take into account only the factors affecting the random uncertainty components: u_c (%) = V_c (%) = $s \cdot \overline{x}^{-1} \cdot 100$, where V_c (%) is the coefficient of variation; s is experimental sample standard deviation, and $\overline{x}$ is the sample mean.

The total measurement uncertainty of whole plants includes random statistical uncertainty calculated from data samples of concentrations of the measured chemicals in leaves, stems and roots of common reed.

The optimum number of samples to be selected in the surveillance location to achieve the desired magnitude of a standard uncertainty of measurement was calculated using the formula [6]:

$$n = \frac{t^2 V^2}{m^2}, \qquad (2)$$

where: t is the table value of t-test (at $P=0,95$, $t=2,0$); m is the set point accuracy of the studies with 3% under these conditions.

The ratio of the concentration of ^{137}Cs, ^{90}Sr, Ca^{2+}, K^{+} in the organs or whole plantof common reed (K_c) was calculated as the relative difference of the measured concentrations of these compounds in the organs:

$$Kc, \% = (C1 - C2) / C2 \cdot 100, \qquad (3)$$

where: C_1 is concentration of a substance in the plant; C_2 is concentration of a substance in the organ, which is compared.

The total uncertainty in the laboratory analysis did not exceed ± 20%. The calculation of statistical sample parameters of data was performed at the confidence probability level 0.95. Assessment of the correlation links of data samples was performed by the coefficient of determination (R^2).

3. Results and discussion

The results of observations show that at a 20 km distance the content of radionuclides in the common reed, selected in aquatic ecosystems, varies by orders of decimal magnitude (Fig. 1).

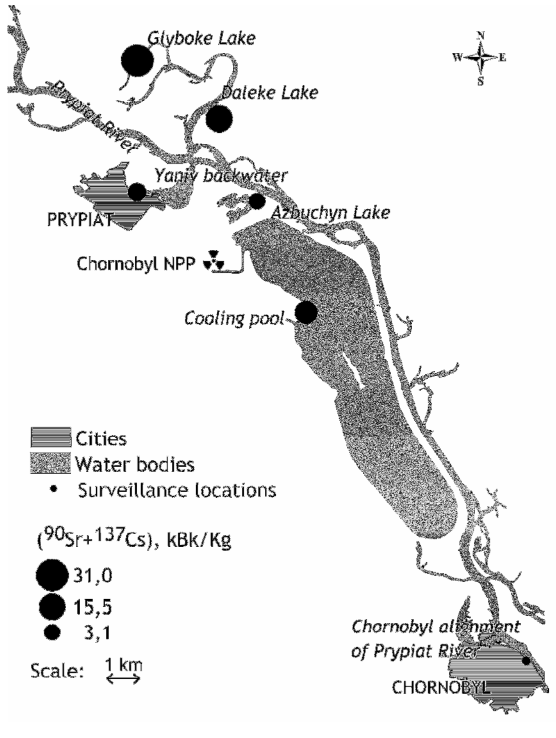

Figure 1. Location of surveillance places in CEZ and common reed leaves total activity (^{90}Sr+^{137}Cs) in 2009.

All surveillance places are found in similar climatic and soil conditions. The banks of surveyed ponds are covered with clay sandy gleying soils with complex long-term, seasonal, and sometimes weather dynamics of groundwater levels, which determines the mobility of radionuclides in the gley geochemical barrier [10].

Throughout the growing season, ^{90}Sr and ^{137}Cs in the catchment area in the surveyed ponds are seasonally affected by climate and weather conditions [11]. For example, 1.3% ^{90}Sr and 64% ^{137}Cs gets in the

Glyboke Lake during spring floods relatively to stock of these radionuclides in the water. During this period, the ratio of $^{90}Sr/^{137}Cs$ in the lake is 0.53. Later, the most part of ^{137}Cs mainly migrates to the slurry falls in the bottom sediments. Most of ^{90}Sr that falls into the lake from the outside is brought by stormwater. The ratio of $^{90}Sr/^{137}Cs$ in water in summer is 26. The correlation between the content of ^{90}Sr and ^{137}Cs in the water during the growing season is missing.

Besides, the migration of radionuclides in water bodies is affected by a variety of man-made load. The ecosystems of the lakes Glyboke and Daleke, in which the radionuclide contamination is maximal, the level of man-made pollution is minimal. Most anthropogenic impact with significant radionuclide contamination has been observed in the ecosystem of the cooling pond of the Chornobyl NPP. Ecosystems of Yaniv backwater and alignment of the Prypiat River near Chornobyl are under the influence of the products of chemical weathering and urban development. These water bodies along with the cooling pond of Chornobyl NPP during the first years after the Chornobyl disaster in 1986 have undergone significant chemical contamination. Under these conditions, the uncertainty of radionuclides measurement in the common reed depends on many factors associated with the condition of the analyzed plants. Among them, the most effective, to our knowledge, are the individual characteristics of radionuclide accumulation common reed and its organs, and also features a seasonal accumulation of radionuclides by plants. Important methodological features of sampling that affect the uncertainties of measurement magnitude are the choice parts of the plant to carry out monitoring and evaluation of the transformation of the samples in the selection and transportation.

*3.1. U*ncertainty of measurement *associated with the individual characteristics of radionuclide accumulation in the common reed*

Assessment of individual heterogeneity of accumulation of chemicals in plants of common reed is held in surveillance places by selection of individual plants (at least five) and the analysis of their parts - leaves, stems and roots. Samples for the analysis of chemicals and radionuclides made on the timing and surveillance location was calculated uncertainty of measurement associated with the individual characteristics of plants are compared with each other (Table 1). The table shows the results of measurements in whole plant preparations calculated on the weight of the crude sample, their roots and aqueous extracts of the leaves and stems (based on the weight of the dry sample). To justify the comparability of measurement results in dry samples and aqueous extracts, magnitude of standard deviations of radionuclide content was compared and result showed their sameness. A correlation analysis showed a close relationship between measurements of ^{90}Sr and ^{137}Cs in dry samples and aqueous extracts with a value of R^2 - 0,80 and 0.87, respectively. The magnitude of the obtained coefficients of determination shows that the results of measurements of gross content of the test substances and their aqueous content in extracts can be compared with the reliability of at least 80%.

The data in Table 1 indicate that the standard deviation of the measured samples in the territory of the main polluting agents is the fact that their radionuclide content exceeds the average by more than 100%. Heterogeneity of radionuclides in samples from the surveillance places, compared to the whole territory - is much lower. The uncertainty of measurement average magnitude for leaves and stems samples common reed collected in separate places reaches 25%.

Table 1. Averaged measurements of substances in organs and whole plants of the common reed (in 6 surveillance locations)

Object	^{137}Cs, kBk/kg	*u, %*	^{90}Sr, kBk/kg	*u, %*	Ca^{2+}, g/kg	*u, %*	K^+, g/kg	*u, %*
Foliage	10±12	23	6,6±7,4	21	2,2±0,9	27	1,1±0,7	23
Stems	6,0±5,6	17	2,1±1,9	13	2,0±1,8	17	1,1±0,3	15
Roots	43±65	69	7,8±9,2	85	-	-	-	-
Whole plant	1,3±1,5	54	0,8±0,9	46	-	-	-	-

Note: The number of measured samples of leaves is 35, stems - 26, roots - 9, whole plants - 21; content of the matter is given by the measurement results of the mixed samples that are average for the entire period of observation; "± 42" is the standard deviation of the substance content in the surveillance place; *u,%* is averaged according to the results obtained in terms of measurements from each surveillance site.

Table 1 shows uncertainty of measurement average magnitude for leaves collected over the entire height of shoots common reed and portions of stems taken at the middle of their height. The same parameter (*u*,%), calculated for the selected leaf shoots at half height for ^{137}Cs and ^{90}Sr is 14% and 12%, respectively. These values are comparable to those calculated for the middle portions of stems (Table 1).

The heterogeneity of the radionuclide content in the roots and whole plants, estimated the magnitude of the uncertainty of measurement is 2.5 times higher compared to the leaves and stems. Comparable with radionuclides, in samples from the field observations, is the uncertainties of measurement magnitude of their chemical counterparts are cations Ca^{2+} and K^+ in leaves and stems. Correlation analysis links the magnitude of the standard deviation to the content in the samples of ^{90}Sr, ^{137}Cs, Ca^{2+}, K^+ showed an average force feedback with the values of R^2 - 0,45, 0,48, 0,35, 0,41, respectively. This means that with an increase of concentrations of these biophilic substances in surveyed ecosystems, their availability to plants is increased, which in resulting in a more uniform accumulation of organisms and reducing the magnitude of the uncertainty of measurement associated with the individual characteristics of accumulation of chemical plants.

The large of the uncertainty of measurement magnitude inherent in the measurement of radionuclides in the roots shows that their study in conducting radiation monitoring may not be widespread because of the large sample magnitude of the corresponding samples required to achieve uncertainties of measurement of less than 50%.

As shown, the correlation analysis of data samples from six surveillance locations, the accumulation of ^{137}Cs and cations K^+, and - ^{90}Sr and Ca^{2+}, common reed leaves is in inverse proportion. Communicate these pairs of substances in the leaves is described value R^2 - -0,25 -0,45 and, respectively. Thus, the uncertainty of measurement, as already mentioned, is reduced as the concentration of the substance. The results indicate a weak non-radiological impact of environmental factors in the field of observation on the magnitude of the uncertainty of radionuclides measurement in samples of common reed.

To ensure the minimum measurement uncertainty it is advisable to use common reed leaves selected at half-height. Also, leaves as the main part of the plant, are preferred as a monitoring tool, as with no additional costs for radionuclide analysis of samples selected from leaves, anatomical, morphological, physiological and other tests can be carried out. In case of stock assessment of radionuclides in biomass of the common reed, the measurement of whole plants is preferred.

To control the uncertainty of measurement under the local background radiation contamination associated with the individual characteristics of radionuclide accumulation by plants or parts thereof, required to select at least five samples of Biological indication. The results of uncertainty of measurement calculated for the sample. The resulting value is attributed to all the composite samples, as related to the individual characteristics of radionuclide accumulation component of the total uncertainty of measurement.

3.2. Uncertainty of radionuclides measurement associated with the peculiarities of their accumulation in plant organs

The problem of unequal accumulation of radionuclides by different organs is relevant only if the entire plant is analyzed. In this case, the magnitude of the uncertainty of measurement depends on the different ratio of mass of leaves, stems and roots of the entire plants selected in different places. Our estimates of the mass of leaves, stems and roots of the selected plants have shown that their ratio can vary significantly depending on season, state of the aquatic ecosystem and sampling conditions. The ratio of the content of radionuclides accumulated in organs of plants in surveillance locations was calculated using formula 3. Given the distribution of ratio of radionuclide accumulation in plant organs, uncertainty of measurement was calculated, which is proportional to mass fraction and the specific activity of the relevant part of the plant selected for analysis (Table. 2).

Table 2. Contribution of organs and the entire plant to the uncertainty of measurement (u, %) of sample mass and specific activity of radionuclides

Object	Mass	^{137}Cs	^{90}Sr
Foliage	23	41	42
Stems	15	20	18
Roots	3	25	8
Sum *u, %*	41	85	68

Studies show that when using composite samples of the entire plants for measurements, the results should be given the uncertainty of measurement magnitudes established for separate organs of such plants calculated in proportion to the ratio of their mass in a sample of the entire plant.

3.3. Uncertainty of measurement related to the peculiarities of seasonal radionuclide accumulation by the plants

During the monitoring of the environment it is important to consider phenological phases of the common reed, because some of them in time coincide with a maximum amount of ^{137}Cs in the water bodies, others – with ^{90}Sr. As mentioned above [10], after the spring floods in the beginning of the growing season in experimental ponds ^{137}Cs dominates. In the period of maximum growth of cane, which takes place in June [11], ^{90}Sr prevail in the water bodies. The concentration of available ^{90}Sr in the water body in August, during the flowering period of the cane, can increase dramatically due to the summer-autumn drought period. These phenomena lead to the need to consider phenological phase of the plant while sampling and to assess uncertainty, which depends on changes of maximum content of radionuclides in the water bodies during the growing period.

The comparison of uncertainty of the radionuclides measurement in the samples of the entire common reed plant and its leaves was made depending on the characteristics of seasonal accumulation of radionuclides. For laboratory analysis the leaves selected at half height of the plants were used. Having as an example the radionuclide content in the leaves of the common reed, which were selected under a local background radionuclide contamination of water bodies in CEZ, one can see that the value of this parameter can be changed by 2 to 3 times by months within the growing period (Fig. 2).

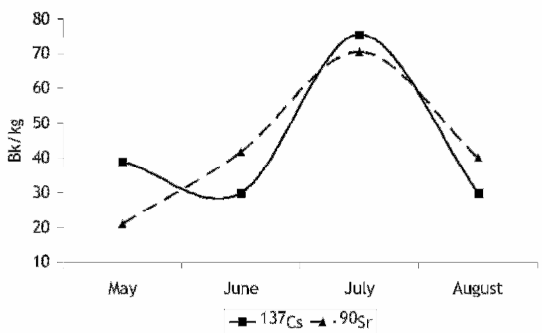

Figure 2. The radionuclides content in the common reed leaves selected from Prypiat River at the alignment of Chornobyl city (averaged over the results of observations of 2009-2013).

Averaged over the surveillance locations value of the variation coefficients in the common reed leaves content of ^{137}Cs and ^{90}Sr varies from 38% to 65% with an average - 51% and from 41% to 89% with an average - 68%, respectively. At the same time, the average uncertainty of measurement associated with the individual characteristics of the relevant radionuclide accumulation in the leaves of certain plants is 23% and 21%, i.e. 2-3 fold less (Table. 1). These values show that the common reed leaves can be used to evaluate seasonal accumulation of radionuclides, as their respective uncertainties of measurement are significantly lower than this parameter. In contrast to the leaves, the entire plants have uncertainty of measurement associated with the individual characteristics of accumulation of radionuclides by ^{137}Cs, more than seasonal one - 54% and 51%, respectively; by ^{90}Sr, comparable to the seasonal one - 46% and 68%, respectively (Table. 1). Such a ratio of uncertainty of measurement does not allow to make a reliable estimation of the seasonal differences of radionuclides accumulation by using the entire plant samples. For reliable evaluation of the radionuclide accumulation by the entire plant samples with available parameters of uncertainties of measurement, several samples in different months of the growing season are required.

3.4. Measurement uncertainly of radionuclides associated with the peculiarities of losing water in plants

Uncertainty of measurement associated with the loss of water by the plant samples during transportation from the site of selection to the laboratory is only relevant in the case of calculating the analysis results on the live weight of plants at the delivery of samples without cooling. Between the sampling and analysis of the entire plants by the first method from 2 to 8 hours passed, while, during the transportation the samples were not cooled. We conducted an experiment to assess the loss of water by leaves of the common reed due to evaporation. From each of the six surveillance locations the leaf samples were taken to the laboratory in cooler bag, in plastic packs, at temperatures of about + 5 ° C. Control weighing showed no significant changes in the weight of the samples with the above conditions of delivery.

To evaluate the losses due to evaporation of water, each sample was placed in desiccators with the presence of a pre-dried calcium chloride at the rate of - 10 sorbent parts per 1 sample part. For 8 hours after 15 min the samples were removed from the desiccators and weighed. On average, the loss of water samples consisted of 17 g/kg/hr, with a variation coefficient of 32%. The greatest loss of water had a sample from the cooling pond Chornobyl NPP ecosystem – 20% of the sample weight within 8 hours. The samples from the Prypiat River ecosystem in alignment of Chornobyl-town had lowest water loss. Since it is impossible to predict the travel time and the initial physiological state of plants at each sampling, we consider it appropriate in this case, to attribute all the results with the maximum uncertainty of measurement is 2.5% for each hour of the path stay from the selection site till the start of measurements.

3.5. Comparison of the quality of measurements using different sampling methods

Calculation of the number of samples to be selected for each of the techniques employed (Table. 3) was conducted on the basis of total uncertainty (forms. 1), the common component of which for both methods is laboratory uncertainty of measurement (20%).

In addition to the laboratory, to calculate the total uncertainty implementing the first method, the following was used: the component related to the loss of water samples during transportation for 4 hours (10%) and seasonal variability of accumulation of radionuclides (^{137}Cs - 51%, ^{90}Sr - 68%). The last parameter includes individual uncertainties and that related to the heterogeneity of the radionuclide content in plant organs. Calculations show that to ensure the indicator accuracy of measurement of 40-45%, about 20 plants must be measured (Table 3). With the indicated uncertainty, significant difference between the measurements from different locations can be set in the case where there is a difference of more than 1.5 times. Furthermore, in studies that implemented the first method, in the established long-term practice, the annual sample data includes measurements of 1-2 whole plants selected monthly, which accounts for 3-4 records a year from one surveillance place. Thus, to achieve the desired reliability of measurement, samples from one surveillance location are not enough.

Table 3. The required number of samples taken from one surveillance location with the given parameters of measurement quality

Object	^{137}Cs		^{90}Sr		Ca^{2+}		K^{+}	
	m	*n*	*m*	*n*	*m*	*n*	*m*	*n*
Foliage	10	46	10	41	10	45	10	40
Stems	10	55	10	44	10	55	10	49
Whole plant	40	16	45	19	-	-	-	-

Note: The calculations have been performed according to the formula 2 (at $P=0,95$, $t=2,0$), using the values of $u,\%$ given in Table 1.

Given these circumstances, the annual data on the content of radionuclides in the samples of common reed (Fig. 1) can be united into three groups on the basis of radionuclide contamination. The first includes samples with background content of radionuclides selected in the ecosystem of the alignment of the Prypiat River near Chornobyl. Second, at the average level of radionuclide contamination of the sample includes samples from the Azbuchyn Lake, Chornobyl NPP cooling pool, Yanivsky backwater (intragroup differences in the content of samples: for ^{137}Cs, K_c = 72% of ^{90}Sr, K_c = 53%). Third, at the maximum pollution level, includes the lake Glyboke and Daleke (the difference between the content of radionuclides in the sample – ^{137}Cs by K_c=60%, ^{90}Sr by K_c=120%). The difference between the content of radionuclides between samples from the group with the highest and average pollution is at ^{137}Cs K_c=250%, and ^{90}Sr

K_c=570%; between samples with the average local background pollution ^{137}Cs K_c=5000%, ^{90}Sr K_c=1400%. Thus, this approach allows the display of the content of radionuclides in the common reed, in case of increase in the number of surveillance locations or the frequency of observations.

Calculating the number of samples selected by the second method produced on the basis of total uncertainty, the components of which are the uncertainty of laboratory measurements and the individual characteristics of radionuclide accumulation (Table 1), showed that the measurement accuracy of leaves and stems is between 5% and 10% if selecting fifty samples from one place (Table 3). The number of samples required for quality assurance measurements of cations Ca^{2+} and K^{+} correspond to that of the radionuclides.

Thus, the performance of measurements by the second method yields quantitative results at comparable costs that are 7 times more accurate as compared with the observations carried out by the first method, which are indicative.

4. Conclusions

Studies have shown that in two methods used at CEZ today for sampling and analyzing the radionuclides content in samples of common reed, uncertainty of measurements is formed differently. Accordingly, the number of components of uncertainty of measurement is different. In the first method of study, when the entire plants are received for analysis and calculation of content of the radionuclides is made on the wet weight of the sample, there are several components of high magnitude of uncertainty of measurement. In this case, the uncertainty of measurement magnitude is so high that to produce a mixed sample in the surveillance location, more than 100 plants are required to ensure satisfactory quality of measurements.

The data available today on the results of long-term series of observations obtained by the first method can be used for indicator evaluation of radioecological parameters of aquatic ecosystems of CEZ. To reduce of the uncertainty of measurement results performed by the first method to group samples from different surveillance places on the basis of the similarity levels of radionuclides in the samples.

Estimation of the value of the total statistical uncertainty of measurement by the second method, which is based on selection of individual plant organs and analytical weight control of the dry samples, showed that in this case a sufficient number of components in a composite sample is 50. The magnitude of uncertainty of measurement in this method is below the difference of radionuclides content in the common reed, even in samples of ecosystems with similar levels of radioactive contamination. This method of uncertainty of measurement estimation allows to investigate the seasonal accumulation of radionuclides by the common reed, and can also be used to monitor the results obtained by the first method.

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