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MNE-ALB
IPA CROSS-BORDER COOPERATION PROGRAMME
MONTENEGRO-ALBANIA 2014-2020

Skadar Lake withOut chemicaL polIUTION - SOLUTION

RESEARCH STUDY ON LAKE SKADAR

- JOINT EXPERTISE
- JOINT PROFFESSIONALS
- JOINT TRAINING
- JOINT RESEARCH
- JOINT DATA EVALUATION
- JOINT EQUIPMENT

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GREEN CENTRE ALBANIA

Impact of Pollutants in Skadar Lake

September 2023

Overall Objective

The specific objective of project is to support the shared and coordinated hazardous chemical substances research in the Skadar Lake cross border area. The project supported establishment of linkage between cross border cooperation and improvement of environmental risk identification mechanisms and knowledge.

This project focussed on the screening of identified hotspots for pollution from Priority Substances and other hazardous compounds. The potential hazards posed by the input of organic micro-pollutants and metals to Skadar Lake have been characterised by **in-depth chemical analysis for priority (hazardous) substances in water, sediment, and biota**.

In addition, a **comparison of the data resulting from EU Water Framework Directive (WFD) compliant chemical analysis and an in vitro bioassay** was carried out validate the effectiveness of the bioassay for future use as a screening tool to provide an indication of the risk for complex mixtures as they occur in surface water and sediment.

What are Priority Substances?

Clean water is essential for healthy ecosystems, as well as for many human uses, including drinking, bathing, and agriculture. Freshwater bodies can be contaminated by a wide range of chemical pollutants. These pollutants are emitted from a variety of sources, including industry, agriculture, transport, mining, and waste disposal, as well as from people's homes. Chemical pollution of surface and groundwater endangers the aquatic environment, with effects such as acute and chronic toxicity in aquatic organisms, accumulation of pollutants in the ecosystem and loss of habitats and biodiversity. It also poses a threat to human health.

Substances, which pose a risk to us and our environment to an extent that priority action must be taken to reduce their occurrence, have been classified as so-called Priority Substances.

Human exposure to hazardous chemicals can occur via ingestion of contaminated water or seafood, or via bathing.

While the risks posed by certain chemicals have long been recognised, new risks from others, either alone or in combination continue to emerge. EU water legislation requires a regular review of the lists of surface water and groundwater pollutants that need to be monitored and controlled.

The WFD list of priority substances identifies the water pollutants that pose the greatest concern and risk to the aquatic environment across the EU, which Member States must monitor in surface waters. Among the 45 chemical pollutants currently categorised as priority substances, including industrial chemicals, pesticides, and metals, 21 are designated as priority hazardous substances, due to their persistence, bioaccumulation, and toxicity.

Under the WFD, measures must be taken to reduce the emissions, discharges, and losses of priority substances into water, and to phase out those of priority hazardous substances, within 20 years of their designation.

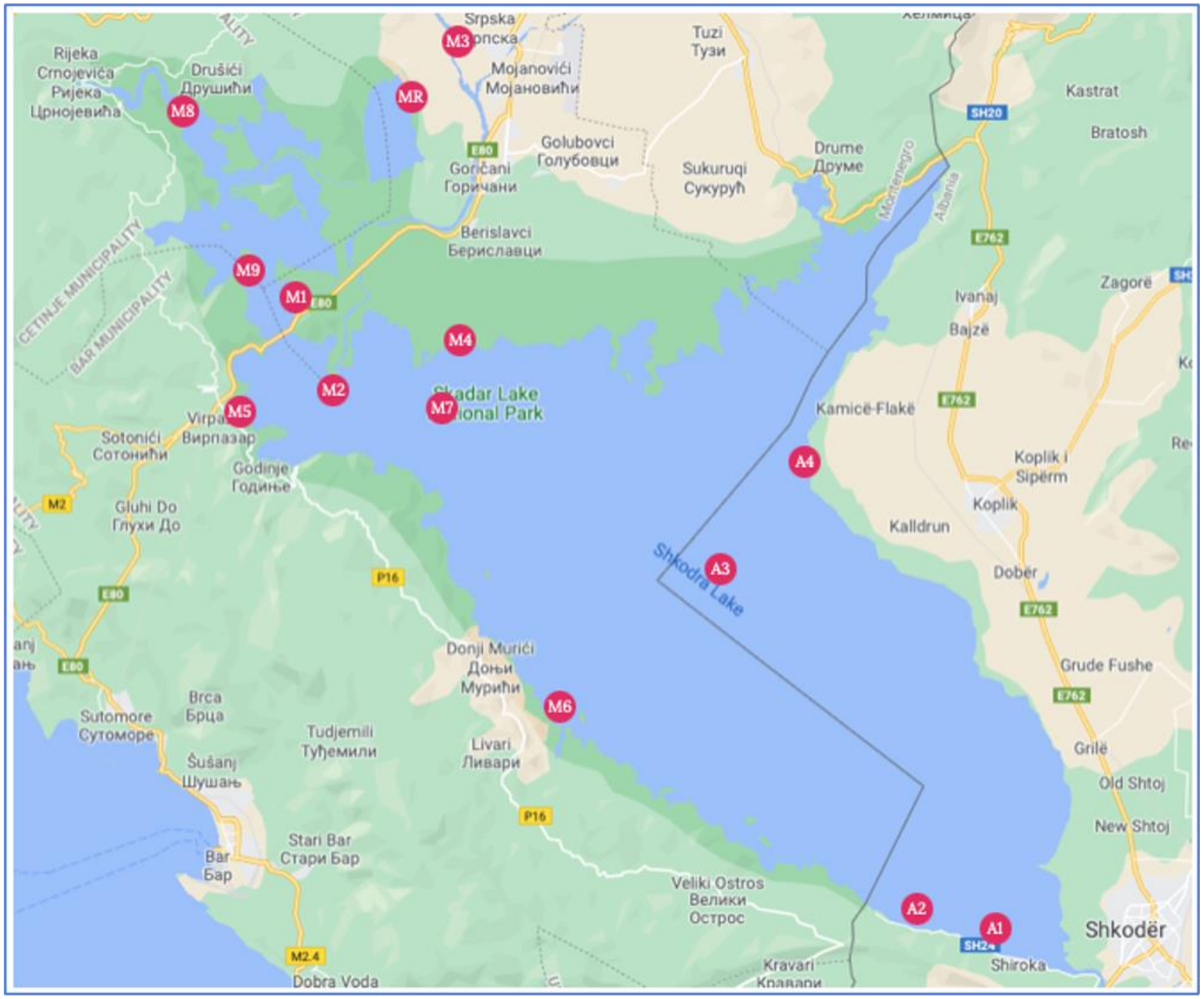
Sampling and Analysis

Montenegro: Ten locations were chosen for the study, seven of which are currently designated in the national water quality monitoring programme. Based on previous water quality analysis it was entirely logical this project to use the same monitoring locations for analysis of Priority Substances in support of the national water quality programme in Montenegro to establish which Priority Substances are present in the water, sediment, and biota at these locations. In addition to the national monitoring programme, a sample location was chosen where two rivers (Orahovština/Crmnička) enter Skadar Lake. Two further sampling sites which are not included in the national monitoring programme, were included to account for the input of pollutants to the main body of water in the west of Skadar Lake which may arise from both the Rijeka Crnojevica and the Morača River. One final location, the Bolje sestre karstic spring discharge area, which is located within the Skadar Lake system, was included, and proposed as a reference site.

Albania: Four locations were chosen for sampling on the Albanian side of the lake to establish the impact of Priority Substances in the water, sediment, and biota. The proposed sampling sites are in line with water quality locations proposed in the Drini-Buna River Basin Management Plan. Thus, data produced in the current study would ultimately act to support the future national water quality monitoring programme. The choice of the locations for sampling sites are based on (i) distance with urbanised and populated areas, (ii) distance with effluents, (iii) distance with draining channels, and (iv) previous scientific data available. One sampling site was in an intensive agricultural area (Sterbeq/Kalldruni) where pesticides are expected to be present. Two sampling sites were located close to urban areas (Shiroka and Zogaj). One sampling site was in the open waters in the middle of Shkodra Lake close to the border with Montenegro.

Sampling locations on the Montenegrin (M) and Albanian (A) side of Skadar Lake

Station No.	Station Name
M1	Morača right, 200 m from the entrance of the right branch of the Morača River into Skadar Lake
M2	Morača left, 500 m from the entrance of the left branches of the Morača River into Skadar Lake
M3	Bistrice, from the river's edge
M4	Skadarsko Plavnica, 200 m from the lakeshore.
M5	Orahovštica/Crmnička, 200 m from the entrance of Orahovštica and Crmnička Rivers into Skadar Lake
M6	Donji Murići, 200 m from the lakeshore.
M7	Lake Skadar Open Waters
M8	Rijeka Crnojevica, 200 m from the river entrance into Skadar Lake
M9	Vučko blato North, middle, near Kamenik
MR	Bolje sestre spring (Reference)
A1	Shiroke (Shkodra), 200 m from the lakeshore
A2	Zogaj (Shkodra), 200 m from the lakeshore
A3	Shkodra Lake, open water in the middle of the Lake
A4	Sterbeq/Kalldruni, 200 m from the lakeshore.



Chemical Analysis of Surface Waters, Sediments and Biota

Chemical analysis of surface waters in the Albanian and Montenegrin sides of the Skadar Lake sub-basin included 44 of the 45 Priority Substances according to the Environmental Quality Standards Directive (EU Directive 2013/39/EU). Additional substances were analysed, which included 6 organochlorine insecticides (Aldrin, Dieldrin, Endrin, Isodrin, DDT and para, para-DDT).

Chemical analysis of sediment collected in the Albanian and Montenegrin sides of the Skadar Lake sub-basin included 18 of the 45 Priority Substances according to the Environmental Quality Standards Directive. Additional substances were analysed accordance with EU WFD CIS Guidance No.25. These included the following classes of chemicals: Polycyclic Aromatic Hydrocarbons, Polychlorinated biphenyls, Dioxin-like polychlorinated biphenyls, Total petroleum hydrocarbons, Organotin compounds, Phthalates, Polychlorinated Dibenzo-p-dioxins, Dibenzofurans, Organochlorinated pesticides, Brominated diphenylethers and Metals.

Chemical analysis of biota was carried out on Carp and Eels collected in the Albanian and Montenegrin sides of the Skadar Lake sub-basin included analysis of 18 of the 45 Priority Substances according to the Environmental Quality Standards Directive. Metals and the following organic compounds have been analysed: Polycyclic Aromatic Hydrocarbons, Polychlorinated biphenyls, Dioxin-like polychlorinated biphenyls, Total petroleum hydrocarbons, Organotin compounds, Phthalates, Dibenzo-p-dioxins, Dibenzofurans, Organochlorinated pesticides and Brominated diphenylethers.

Environmental Quality Standards

Quality Standards in Surface Waters

The Environmental Quality Standards Directive lays down EU-wide environmental quality standards for the 45 priority substances listed in Annex X the EU Water Framework Directive as well as eight other pollutants that were already regulated at EU level before Annex X was introduced in 2001.

Two types of environmental quality standards are set for priority substances in the EQSD: annual average concentrations (AA-EQS) and maximum allowable concentrations (MAC-EQS). The former protects against long-term chronic pollution problems, and the latter against short-term acute pollution.

Good surface water chemical status means that the concentrations of all priority substances do not exceed the environmental quality standards. Status assessments are performed every 6 years.

Quality Standards in Sediments and Biota

For monitoring, sediment and biota are the most suitable matrices for many substances because they integrate, in time and space, the pollution in a specific water body. However, relatively few of the Priority Substances have common Environmental Quality Standards for sediment or biota, since these are required to be based on national selection, which is a costly and time-consuming process.

To assess the impact of Priority Substances and other hazardous substances in sediment and biota in Skadar Lake, environmental quality standards used from the NORMAN database, which based on the experience of a world-wide network of laboratories.

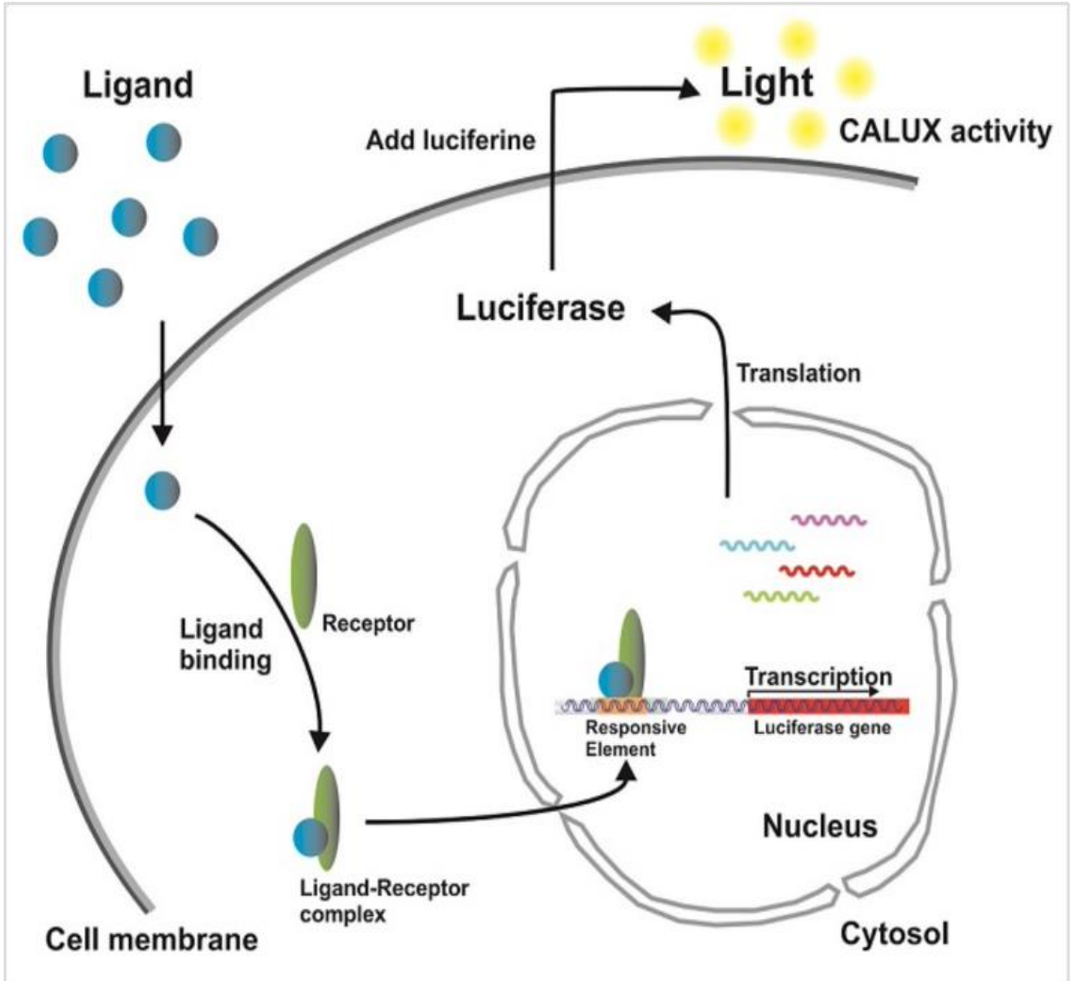
The NORMAN database provides Predicted No Effect Concentrations (PNEC) to a range of biota for all chemicals evaluated in this study. The extent to which chemicals exceed the PNEC provided a clear indication of the magnitude of the pollution.

Procedure for in vitro bioassay

The commercial in vitro CALUX (Chemical Activated Luciferase Gene Expression) bioassay is based on a reporter gene approach and the assays produce light when exposed to substances that induce certain pathways. The assays form a panel of mammalian cell lines that were modified to produce a quantifiable response in addition to the natural response. The molecule-receptor complex binds to specific DNA sequences (called “responsive elements”), triggering the expression of certain genes, in turn giving rise to the toxicological response. If a cell is triggered to generate a response, a reporter gene (luciferase) is transcribed and translated into an enzyme that produces light during a reaction it catalyses. After addition of a substrate (luciferine), light production is quantified using a luminometer where the produced signal is proportional to the evoked effect.

Samples of sediment were taken from 8 locations in Skadar Lake (Montenegro). One further sample of sediment was collected upstream in the Morača River. The Bolje sestre karstic spring discharge area, which is located within the Skadar Lake system, was included in the sampling programme, and proposed as a reference site. Two in vitro CALUX bioassays were compared to the results obtained by chemical

CALUX Assay Principle



Name of Assay	Bioactive Compounds
PAH CALUX	Carcinogenic PAHs (such as Benzo(a)pyrene)
ER CALUX	Estrogens, EDCs, Bisphenol A, Phthalates, Pesticides, Pharmaceuticals, cosmetics

Results

Surface Waters

Montenegro: The analysis of Priority Substances was carried out at 10 separate locations, on three separate sampling occasions during the period October 2021 to June 2022. The results of the analysis showed that for all locations, Priority Substances (or any additional insecticides) did not exceed, the MAC-EQS values in surface waters. Levels above the PNEC values were however observed for the organophosphorus insecticide chlorpyrifos and polycyclic aromatic hydrocarbons (PAHs).

Albania: The analysis of Priority Substances in surface waters was carried out at four locations, on three separate sampling occasions during the period October 2021 to June 2022. The results of the analysis clearly showed that Priority Substances (or any additional organochloride insecticides) did not exceed either the MAC-EQS or the PNEC values.

Sediment

Montenegro: On the Montenegrin side of the lake, the analysis of sediments was carried out at 10 locations, on two separate sampling campaigns in October 2021 and June 2022. The results of the analysis showed that substances within the same three classes of compounds were measured above the PNEC values. These included 7 PAHs , which included (Benzo(b)fluoranthene, Benzo(k)fluoranthene, Benzo(g.h.i)perylene, Benzo(a)pyrene, Chrysene, Fluoranthene, Indeno(1.2.3-cd)pyrene), 2 organochlorinated pesticides (DDD and DDE) and 5 metals (Cadmium, Chromium, Manganese, Nickel and

Albania: The analysis of sediments was carried out at on the Albania side of the lake at four locations, on two separate sampling campaigns in October 2021 and June 2022. The results of the analysis indicate that substances within three classes of compounds were measured above the PNEC values. These included 6 PAHs which included (Benzo(k)fluoranthene, Benzo(g.h.i)perylene, Benzo(a)pyrene, Chrysene, Fluoranthene, Indeno(1.2.3-cd)pyrene), one organochlorinated pesticide (DDD) and one metal (Manganese).

Biota

The results of the chemical analysis of Carp and Eels showed that one organochlorinated pesticide (DDE) and one metal (mercury) were measured above the EU Environmental Quality Standard and/or PNEC values.

The concentration of DDE in eels on both sides of the lake were measured 1.5 to 2.5 times above the PNEC.

The concentration of mercury in carp sampled on the Albanian side of the lake was slightly over EU environmental quality standard, whereas mercury measured in carp caught on the Montenegrin side was over 5-fold above the standard. The concentration of mercury measured in eels was significantly higher, ranging from 4.5 times and 8.0 times greater than the EQS (WFD) for species taken from the Albanian and Montenegrin sides of the lake, respectively.

It must be stressed however that comparison with the norms from Commission Regulation (EC) No 1881/2006, which dictates the maximum levels for contaminants in foodstuffs, showed that all contaminants that are measured in accordance with the legislation in this type of sample are well below the maximum levels for organochlorinated pesticides and mercury.

Comparison of CALUX Bioassay with Chemical Analysis

Current chemical monitoring approaches under the EU Water Framework Directive (WFD) focus on certain substances identified as priorities, but they do not consider other potentially harmful substances and ignore the hazards related to contaminant cocktails.

Bioassays (effect-based tools) are a potential solution for assessing such complex samples. The ability to detect toxicity and provide needed biological context, providing some measure of risk in terms of the potential for an adverse effect.

The main advantages of in vitro bioassays are the ability to be performed on many different matrixes, such as surface water, sediment, biological tissues, and effluents, together with their ability to detect the cumulative toxicity of mixtures of both known and unknown chemicals in a sample.

The use of bioassays has been mentioned in the context of the WFD in the Common Implementation Strategy (CIS) guidance on water chemical monitoring, sediment, and biota monitoring, and in relation to sediment assessment on environmental quality standards.

A comparison of the data resulting from an in vitro CALUX bioassay and WFD-compliant chemical analysis for PAHs was primarily carried out to validate the effectiveness of the bioassay for use as a screening tool for the identification of polycyclic aromatic hydrocarbons (PAH) in the sediment.

The results provided by the *in vitro* bioassay showed that PAH contamination of the sediment is evidenced in all samples and clearly concur with the results obtained by chemical analysis.

Comparison of the concentration of PAH in sediment determined by in vitro bioassay and chemical analysis.

Station No.	CALUX Bioassay Total PAH (ug/kg dry weight)	Chemical Analysis Total PAH (ug/kg dry weight)	Ratio
M1	540	134	4.0
M2	15,000	2,617	5.7
M4	3,600	91	3.9
M5	1,000	191	5.2
M6	1,600	355	4.5
M7	1,800	425	4.2

Recommendations

The following recommendations are based on the results of the study.

- Calculate the Annual Average EQS values of Priority Substances in all designated surface water bodies in the Skadar Lake sub-basin. This is in line with the requirement by the EU WFD to undertake analysis of a minimum of 12 samples for each designated surface water body within a period of one year during the river basin planning cycle (6 years).
- Undertake monitoring and analysis of all key discharges into the lake sub-basin to calculate the loading and entry points of Priority Substances and metals to the lake.
- A battery of CALUX bioassays are recommended for future screening of sediments, surface waters and discharge effluents in the Skadar sub-basin. The selection of bioassay types was made because a) they were all previously selected for toxicity characterisation studies in surface waters, and b) that they provide the means to screen for many of compounds included and proposed for inclusion in the EU Watch List.

- Due to high natural background levels of some metals, it is important to determine the EQS for individual metals in the lake sediment.
- Develop an investigative monitoring programme for surface waters in the Morača River (and its tributaries) and in groundwaters connecting to the Morača River and Skadar Lake to locate the source of the broad-spectrum organophosphorus insecticide, chlorpyrifos.
- Develop and undertake an investigative monitoring programme to confirm the location of use of the banned organochlorine pesticide DDT in the Skadar Lake sub-basin.
- Establish continuous research of the most frequently consumed fish samples from Lake Skadar for the content of contaminants for which there are standardised .maximum limits in accordance with the legislation on food safety.