UDK/*UDC*: 502.51:543.2(083.74)(497.4)
Izvirni znanstveni članek – *Original scientific paper*
DOI: 10.15292/acta.hydro.2023.02

Prejeto/*Received:* 21.02.2023
Sprejeto/*Accepted:* 29.09.2023
Objavljeno na spletu/*Published online:* 12.01.2024

BIOAVAILABILITY-BASED ENVIRONMENTAL QUALITY STANDARDS FOR METALS UNDER THE WATER FRAMEWORK DIRECTIVE

OKOLJSKI STANDARDI KAKOVOSTI ZA KOVINE V OKVIRU VODNE DIREKTIVE Z UPOŠTEVANJEM NJIHOVE BIORAZPOLOŽLJIVOSTI

Mojca Durjava,[1*] Boris Kolar[1]

[1] National Laboratory of Health, Environment and Food, Prvomajska 1, 2000 Maribor, Slovenia

Abstract

Metals are naturally present in surface waters. By considering the bioavailability of a metal in an aquatic environment, we can more precisely predict the effect of a selected metal on aquatic organisms. This enables us to make a more reliable assessment of the compliance of the metal's concentration in inland surface waters with the environmental quality standard for that metal. In addition to complex-forming inorganic and organic ligands, the bioavailability of metals in water is also influenced by physicochemical conditions in the water. Metals occur in different concentrations and in different forms in aquatic environments. This is referred to as metal speciation. The bioavailability of a metal in water depends mainly on its speciation. Normally, a dissolved free metal ion is more bioavailable than a metal ion bound in a complex. The risks posed by given metals to aquatic environments are addressed under EU legislation in the Water Framework Directive (Council of the European Union, 2000) (WFD). The WFD's purpose is to direct Member States to protect and improve the status of all water bodies throughout the European Union. Directive 2013/39/EC (Council of the European Union, 2013), which refers to priority substances as regards water policy, supplements the WFD and the Directive on environmental quality standards (Council of the European Union, 2008). For nickel and lead, Directive 2013/39/EC sets the annual average environmental quality standards (AA-EQS) for inland surface waters, which refer to biologically available concentrations of metals ($AA\text{-}EQS_{bioavailable}$). In this way, uniform environmental quality standards for the entire European Union are determined for metals, allowing for a compliance assessment of metals for surface waters. In the article, we have presented an analysis of inland surface water monitoring data for nickel and lead, examining how the introduction of $AA\text{-}EQS_{bioavailable}$ affects the consistency of monitoring data for these metals. We used monitoring data from 2018 to 2021. To calculate bioavailable concentrations for nickel and lead, we used the bioavailability assessment model bio-met bioavailability tool v.2.3 (Bio-met, 2017). Additionally, for lead, we used equations (1), (2), and (3) to calculate the bioavailable concentration (European Commission, 2019). Based on the analysis conducted, we recommend the utilization of the bio-met model instead of the equations for calculating the concentration of bioavailable lead in the context of the annual compliance assessment of inland surface water monitoring data

[*] *Correspondence* / Stik: mojca.durjava@nlzoh.si

carried out by ARSO. Using the mentioned bioavailability assessment model, we have provided an upgrade in the methods used to assess metal's concentration compliance with the EQS for that metal for surface waters. As a result, the requirements of Directive 2013/39/EC relating to biologically available concentrations of metals could be transferred to the Slovenian legal system.

Keywords: bioavailability of metals, monitoring, environmental quality standards, surface waters, Water Framework Directive.

Izvleček

Kovine so naravno prisotne v površinskih vodah. Z upoštevanjem biorazpoložljivosti izbrane kovine v vodnem okolju lahko bolj natančno predvidimo, kakšen učinek ima kovina na organizem. To nam omogoča, da izvedemo bolj zanesljivo oceno skladnosti koncentracije kovine v notranjih površinskih vodah z okoljskim standardom kakovosti za to kovino. Na biorazpoložljivost kovine v vodi poleg anorganskih in organskih ligandov, ki tvorijo komplekse, vplivajo tudi fizikalno-kemijski pogoji v vodi. Kovine se v vodnem okolju pojavljajo v različnih koncentracijah in oblikah. To se imenuje speciacija kovin. Biorazpoložljivost kovine v vodi je odvisna predvsem od speciacije. Običajno je raztopljeni prosti ion kovine bolj biorazpoložljiv kot ion kovine, vezan v kompleks. Tveganja, ki jih kovine predstavljajo za vodno okolje, so v okviru zakonodaje EU obravnavana tudi v Vodni direktivi (Council of the European Union, 2000), katere naloga je usmerjanje držav članic pri izboljšanju oziroma ohranjanju stanja vseh vodnih teles znotraj EU. Direktiva 2013/39/ES (Council of the European Union, 2013) v zvezi s prednostnimi snovmi na področju vodne politike dopolnjuje Vodno direktivo in Direktivo o okoljskih standardih kakovosti (Council of the European Union, 2008). Direktiva 2013/39/ES za nikelj in svinec okoljski standard kakovosti za letno povprečje (LP-OSK) v celinskih površinskih vodah zamenjuje z OSK za biološko razpoložljive koncentracije (LP-$OSK_{biorazpoložljiv}$). Na ta način je za celotno Evropsko unijo predpisan enotni okoljski standard kakovosti. To omogoča enotno ocenjevanje skladnosti za kovine za vodno okolje. V članku smo prikazali analizo podatkov monitoringa celinskih površinskih voda za nikelj in svinec ter preverili, kako uvedba LP-$OSK_{biorazpoložljiv}$ vpliva na skladnost podatkov monitoringa za ti kovini. Pri tem smo izhajali iz podatkov monitoringa med letoma 2018 in 2021. Za izračun biorazpoložljivih koncentracij za nikelj in svinec smo uporabili model za oceno biorazpoložljivosti bio-met bioavailability tool v.2.3 (Bio-met, 2017). Za svinec smo za izračun biorazpoložljive koncentracije uporabili tudi enačbe (1), (2) in (3) (European Commission, 2019). Na osnovi izvedene analize priporočamo, da se za letno oceno skladnosti spremljanja celinskih površinskih voda, ki jo izvaja ARSO, namesto enačb za izračun koncentracije biološko razpoložljivega svinca uporabi model bio-met. Z uporabo omenjenega modela za oceno biorazpoložljivosti smo na področju vrednotenja okoljskih standardov kakovosti za kovine in njihove spojine zagotovili nadgradnjo ocene skladnosti kovin za površinske vode. To je omogočilo prenos zahtev Direktive 2013/39/ES, ki se nanašajo na biološko razpoložljive koncentracije kovin, v slovenski pravni red.

Ključne besede: biorazpoložljivost kovin, monitoring, okoljski standardi kakovosti, površinske vode, Vodna direktiva.

1. Introduction

Metals can occur naturally in aquatic environments or enter a water body through industrial production, product use, or recycling. The risks metals pose to aquatic ecosystems are addressed in the chemical legislation REACH (The European Parliament and the Council of the EU, 2007) and the Water Framework Directive (Council of the European Union, 2000) (WFD) within the framework of EU legislation. When determining environmental quality standards (EQS) within WFD, some special features of metals should be considered:

- Metals are naturally present in surface waters.
- A metal's toxicity for aquatic organisms depends on its bioavailability, which depends on its speciation.
- Metal speciation depends on the water's physical and chemical conditions.

- Some metals, e.g. iron, zinc, copper, selenium, manganese, chromium, and cobalt, are essential for the functioning of biological systems.

Under Directive 2013/39/EC (Council of the European Union, 2013), the EQS parameter expressed as an annual average value (AA-EQS) for nickel and lead for inland surface waters are determined, referring to biologically available substance concentrations ($AA\text{-}EQS_{bioavailable}$), with the aim of providing a uniform approach to the derivation of AA-EQS values for metals for the entire EU.

$AA\text{-}EQS_{bioavailable}$ values are determined by considering the "worst-case scenario", which is one of the key principles behind assessing chemical risks. Furthermore, they refer to the most sensitive conditions in surface waters, the conditions where the bioavailability of the metal is the largest.

2. Materials and methods

2.1. Bioavailability of metals in water

Bioavailability is a term that is used in several scientific fields and therefore has many definitions. For metals and their EQS under the WFD, the agreement applies that bioavailability is a combination of the physicochemical factors that influence the metal's behavior (abiotic part) and the nature of the biological receptor (biotic part). This combination results in the specific physiological effects influenced by the route of entry and the duration and frequency of exposure. By considering a metal's bioavailability in an aquatic environment, it is easier to predict that metal's effect on organisms, and so assessment of metal's concentration compliance with the EQS for that metal for inland surface waters can be conducted more realistically.

A metal's ecotoxicological effect in an aquatic environment often cannot be assessed solely from its total concentration in the water, nor from the concentration of the metal dissolved in the water. Metals occur in various concentrations and forms in aquatic environments. This is referred to as metal speciation. A metal's bioavailability in water mainly depends on its speciation, with a dissolved free metal ion usually more bioavailable than a metal ion bound in a complex (European Commission, 2019). It has been proven that the formation of organic or inorganic metal complexes in most cases reduces the metal's bioavailability and thus its toxicity for aquatic organisms (European Commission, 2019). In addition to the presence of inorganic and organic ligands that form complexes, the bioavailability of metals in water is influenced by pH and redox potential, concentrations of the main cations, alkalinity, and ionic strength (European Commission, 2019).

2.2. Models for calculating bioavailability

Several speciation models are currently available for the characterization of chemical species in solution with inorganic and organic ligands (e.g. the Windermere Humic Aqueous Model WHAM, the geochemical modeling software MINTEQ, and the biotic ligand model BLM) (European Commission, 2019; Merrington, 2015). While WHAM and MINTEQ are valuable tools for assessing a metal's behavior in specific contexts (humic-rich environments for WHAM and geochemical equilibria for MINTEQ), the focus of the WFD is broader and encompasses a range of aquatic environments (European Commission, 2019). The BLM's applicability to various freshwater systems and its emphasis on bioavailability make it a more versatile choice for regulatory assessments under the WFD. The BLM is specifically designed to address metals' bioavailability to aquatic organisms. It provides insights into how metals interact with organisms and helps assess the actual risk to aquatic ecosystems. Its incorporation into the WFD ensures consistency and harmonization with established practices and allows for comparisons across different regions and countries. The Biotic Ligand Model (BLM) was developed and applied to a variety of metals, with a primary focus on those that are commonly found in aquatic environments and have the potential to impact aquatic organisms (e.g. copper, zinc, silver, nickel, and lead) (Cousins et al., 2009). With the help of either validated biotic ligand models or their simplified versions (i.e. user-friendly models for /assessing bioavailability), site-specific risk can be assessed. User-friendly models are incorporated into the WFD's assessment and

management processes to assess the compliance of monitoring data (European Commission, 2019; Merrington, 2015).

2.2.1. Biotic ligand model

The biotic ligand model (BLM) (Santore et al., 2001) is based on the principle of chemical equilibrium, which takes into account the interactions of metal, hydrogen, calcium, and magnesium ions with natural organic substances in the aqueous phase. Taking all the above factors into account, the speciation of a specific metal can be calculated (Figure 1, step 1). A biotic ligand, which is in fact an aquatic organism, is seen as an additional ligand in the system (Figure 1, step 2). The model's concept is shown in Figure 1, where hydrogen, sodium, calcium, and magnesium ions compete with the free metal ion for binding sites on the biotic ligand, and thus affect the metal's level of toxicity for an exposed organism. The toxicity is in direct proportion to occupied binding sites on the biotic ligand: the more hydrogen, sodium, calcium and/or magnesium ions there are in the water, the lower the probability that the metal ion will bind to the biotic ligand. In such conditions the metal is less toxic. In other words, the fewer of the aforementioned ions there are in the water, the more the metal ion wins when binding to the biotic ligand, resulting in a more pronounced toxic effect on the organism.

In addition to ions present in water, toxicity is also influenced by organic substances (dissolved organic carbon, DOC). The higher the DOC concentration, the lower the number of available sites on the biotic ligand and the lower the metal's toxicity of the metal.

The BLM speciation model uses site-specific physical and chemical data to estimate the proportion of the measured metal that is biologically relevant, and that can therefore cause toxic effects. Thus, we can obtain the metal's bioavailable concentration in water at the location, which can then be used to assess compliance with the environmental quality standard for that metal (AA-$EQS_{bioavailable}$).

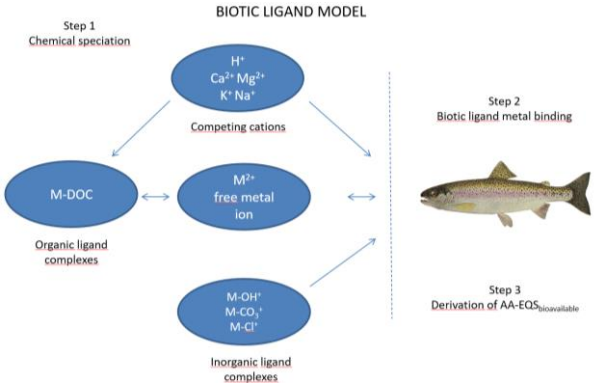

Figure 1: *Schematic representation of the biotic ligand model (Adapted from Vink and Verschoor, 2010).*

Slika 1: *Shematski prikaz modela biotskega liganda (povzeto po Vink in Verschoor, 2010).*

BLM models have been tested and validated both in the laboratory and in the field using large amounts of data, so they are reliable (European Commission, 2019). So far, chronic toxicity models for inland waters have been developed for copper, zinc, silver, nickel, and lead (Cousins et al., 2009). For cadmium, the use of a bioavailability factor that considers water hardness was proposed and implemented within the WFD in order to evaluate bioavailability for chronic toxicity (Council of the European Union, 2013). For lead, the use of a bioavailability factor that considers the concentration of dissolved organic carbon (DOC) was proposed for evaluating bioavailability for chronic toxicity and implemented within the WFD (see Chapter 2.2.3).

When using BLM models, it should be considered that they were developed and validated for a certain range of parameter values that have the greatest impact on the bioavailability of metals (pH, DOC, Mg2+, Ca2+, CaCO3). These parameters should be considered in order to ensure reliable calculation of the bioavailable concentration of a specific metal in water.

Figures 2 and 3 show a summary of BLM, along with other relatively simple ways of calculating bioavailable concentrations of lead, copper, zinc, nickel, and cadmium, as well as the influence of DOC, ions, and water hardness on the bioavailability of a particular metal.

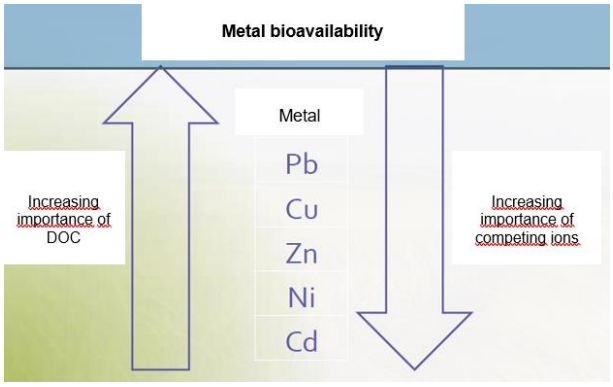

Figure 2: *Bioavailability of metals and the influence of DOC concentration and ion concentration (Adapted from Peters, 2011).*

Slika 2: *Biorazpoložljivost in vpliv koncentracije DOC ter koncentracije ionov (povzeto po Peters, 2011).*

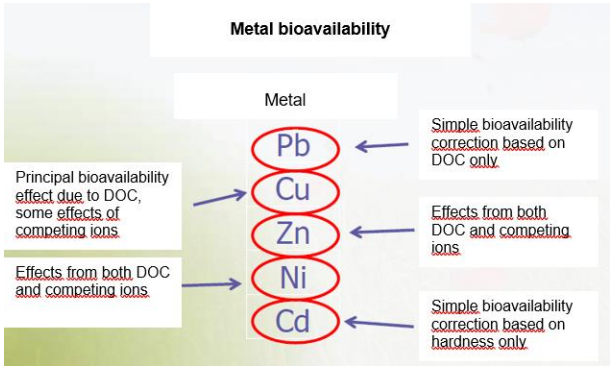

Figure 3: *Bioavailability of metals and the influence of DOC, ions, and hardness on the calculation of bioavailability (Adapted from Peters, 2011).*

Slika 3: *Biorazpoložljivost kovin in vpliv DOC, ionov ter trdote na izračun biorazpoložljivosti (povzeto po Peters, 2011).*

2.2.2. User-friendly models for calculating the bioavailability of metals

The biggest drawback of the BLM models developed so far is that they are too complicated for routine use (European Commission, 2019). Therefore, user-friendly BLM-based bioavailability models have been developed in order to assess the compliance of metal monitoring data with $EQS_{bioavailable}$. These models allow for easy calculation of the site-specific bioavailable concentration of a metal in water using only a few input data (European Commission, 2019). These simplified models imitate BLM models, work in MS Excel, can process thousands of data at once, provide an easy overview of the calculations, and are validated using BLM models (European Commission, 2019).

Three user-friendly models, developed respectively for copper, zinc, and nickel in inland surface waters are currently available: the bio-met bioavailability tool (www.bio-met.net), M-BAT (https://www.wfduk.org/resources/rivers-lakes-metal-bioavailability-assessment-tool-m-bat), and PNEC-pro (www.pnec-pro.com) (European Commission, 2019). When choosing a model for calculating the value of bioavailable concentrations in water at selected location, we considered that the M-BAT tool is largely based on the bio-met ecotoxicity databases, and the M-BAT and bio-met tools both generate relatively similar results (Peters et al., 2016). We also considered that the bio-met and M-BAT models have fewer false positive values than the PNEC-pro model (Merrington, 2015). False positive values appear due to the program's statistical shortcomings, and may result in lower values of calculated bioavailable concentrations (Sokal and Rohlf, 1995). This is not acceptable, as it leads to an underestimation of the risk that a metal poses for an aquatic ecosystem.

Based on the collected data on available bioavailability models, the authors of this paper recommended that the bio-met bioavailability tool (www.bio-met.net) be used by the Slovenian Environmental Agency (ARSO) to calculate the bioavailable concentrations of nickel from measured concentrations, in the context of the annual compliance assessment of inland surface water monitoring data. The decision to use the bio-met bioavailability tool is grounded in its reliable ecotoxicity databases, its similarity in results with M-BAT, and its demonstrated reduction in false positive values when compared to the PNEC-pro model. The site-specific bioavailable concentration of metals is calculated and used for comparison with $AA\text{-}EQS_{bioavailable}$, as shown in Figure 4.

Bio-met is a free online resource for determining compliance with the EQS derived for metals in freshwater aquatic environments, as determined in

the WFD. It is a tool based on a "lookup-table", in which results are matched with nearby results from full-BLM calculations. The tool was developed by WCA Environment from Great Britain, and ARCHE Consulting from Belgium. The latest version (v5.1), which was launched in 2022, allows users to calculate the bioavailable concentrations of lead, nickel, copper, zinc, and cobalt.

The basic approach behind the bio-met tool is based on a large database of more than 20,000 different combinations of key input parameters (concentrations of copper, zinc, nickel, pH, DOC, and concentration of calcium ions) and the corresponding 5% hazardous concentration (HC5) calculations for various metals (Ni, Zn, Cu and Pb), using their respective validated BLM models. The concentration of HC5 is calculated based on ecotoxicological chronic toxicity data where a large amount of data is available, using the SSD statistical method (van Vlaardingen et al., 2004)

To calculate a metal's bioavailability of a metal for a given location, the measured metal concentration, pH, DOC, and calcium ion concentration is input into the model.

When using user-friendly models, it is important to consider the application range,, just as it is for BLM models. The application range of the bio-met model is specified for pH, DOC, and calcium ions.

2.2.3. Bioavailability calculation tools developed for cadmium and lead

To calculate the annual average of the site-specific bioavailable concentration of lead and cadmium, equations are available that consider different conditions in the water. No tool is available for other metals and organometallic complexes (mercury, tributyl tin compounds).

For cadmium it is necessary to consider water hardness. The EQS Directive (Council of the European Union, 2013) provides AA-EQS values for cadmium according to water hardness for four different types of water. To calculate the average annual bioavailable concentration of lead, a simple model consisting of three equations is available in the instructions for considering the bioavailability of metals in water (European Commission, 2019), and in the data on the calculation of EQS for lead (United Kingdom and SCHER, 2011). The model considers that the annual average bioavailable concentration of lead depends on the concentration of dissolved organic carbon (DOC). When using the model, it is necessary to consider the model's application range (DOC, pH, and water hardness).

$$\text{AA–EQS}_{location} = \text{AA–EQS}_{bioavailable} + 1{,}2x(DOC - DOC_{reference}) \quad (1)$$

where AA-EQS$_{location}$ is the calculated environmental quality standard at the location in µg/L. AA-EQS$_{bioavailable}$ is the bioavailable environmental quality standard (Council of the European Union, 2013) in µg/L. DOC is the dissolved organic carbon concentration in mg/L. DOC$_{reference}$ is the average value of DOC in ecotoxicological tests, which is 1 mg/L.

Using equation (1), based on the conditions at the location (i.e. the DOC value), the environmental quality standard typical for the site where the water sample was taken (i.e. the AA-EQS$_{location}$ value) is determined. Then, using equation (2), the unitless bioavailability factor (BioF), which is the ratio between AA-EQS$_{bioavailable}$ and AA-EQS$_{location}$ (United Kingdom and SCHER, 2011), is calculated.

$$BioF = \text{AA–EQS}_{bioavailable} / \text{AA–EQS}_{location}) \quad (2)$$

where BioF is unitless bioavailability factor. The BioF value is less than or equal to 1. When it is equal to 1, this means that lead is 100% bioavailable under site conditions, and such a site is described as a site with "sensitive conditions". Finally, equation (3) calculates the bioavailable lead concentration at the location, specifically by multiplying the value of the measured lead concentration with the BioF value:

$$\text{AA}_{bioavailable\ location} = \text{measured}_{dissolved\ location} \text{ x BioF} \quad (3)$$

where AA$_{\text{bioavailable location}}$ is the annual average bioavailable concentration at the location, and measured$_{\text{dissolved location}}$ is the measured dissolved concentration at the location.

3. Results and discussion

3.1. Assessing the compliance of bioavailable metals concentrations in Slovenia's inland surface water monitoring data with AA-$EQS_{bioavailable}$

The EQS directive (Directive 2013/39/EC) introduced bioavailable risk limits AA-$EQS_{bioavailable}$ for nickel and lead in inland surface waters; limits lower than the previous AA-EQS for these two metals. AA- $EQS_{bioavailable}$ are 4 µg/L and 1.2 µg/L for nickel and lead, respectively. Our survey reviewed river condition monitoring data for nickel and lead and their compliance with AA-$EQS_{bioavailable}$ during the period 2018-2021, and checked how the introduction of AA-$EQS_{bioavailable}$ affects the chemical state of rivers. To gain insight into non-compliant measurement sites, we checked the annual averages of nickel and lead measurements against AA- $EQS_{bioavailable}$ values. A measuring site is non-compliant in a given year if the annual average of measured metal concentrations in its water is greater than the AA-$EQS_{bioavailable}$ values.

The compliance assessment of inland surface water monitoring data presented on Figure 4 is based on comparison between the bioavailable concentration of the metal in the water and the AA- $EQS_{bioavailable}$ values. Local bioavailable metal concentrations at a given site were calculated from the total dissolved concentrations measured.

To calculate bioavailable concentrations, we followed the instructions for the assessment of bioavailability (European Commission, 2018), and the instructions presented in the document on the determination of AA-$EQS_{bioavailable}$ for lead (United Kingdom and SCHER, 2011). For nickel, copper, and zinc, a bio-met bioavailability tool based on BLM was used, and for lead equations (1), (2) and (3), a bioavailability tool based on BLM was used.

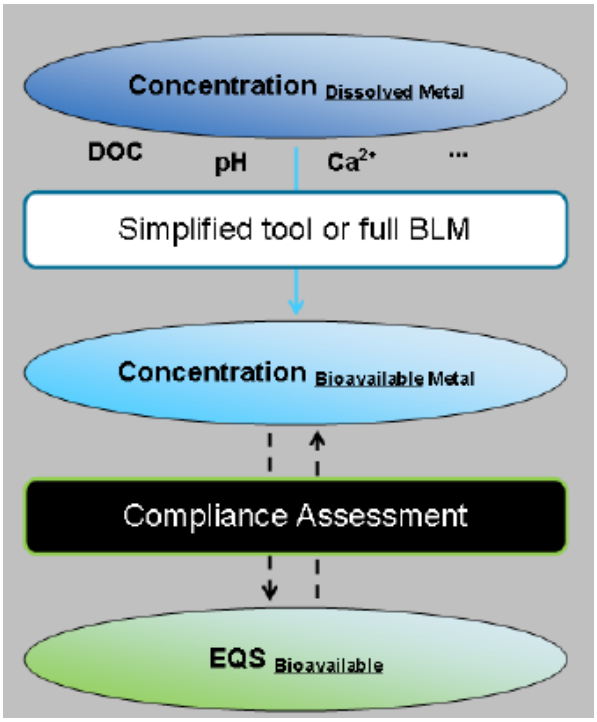

Figure 4: *Compliance assessment of inland surface water (European Commission, 2019).*

Slika 4: *Ocenjevanje skladnosti celinskih površinskih voda (European Commission, 2019).*

The bioavailable concentration of a metal is generally lower than the total dissolved concentration because only a fraction of it will usually be bioavailable, unless the water's physicochemical conditions result in high levels of bioavailability (European Commission, 2019). Under conditions of high bioavailability, the water chemistry conditions will be considered "sensitive conditions".

Slovenia's national inland surface water monitoring data (rivers and lakes) between the years 2018 and 2021, as provided by ARSO, were analyzed in two steps according to the "Technical guidance for implementing EQSs for metals" (European Commission, 2019):

Step 1: Checking the consistency of the measured metal concentrations.

ARSO checked the consistency of river condition monitoring data for the period 2018-2021, using data from the inland surface water monitoring database. More specifically, they evaluated the consistency of the annual averages of nickel and lead measurements with AA-$EQS_{bioavailable}$ values

for these metals. 43 measurements from 19 sampling sites exceeded AA-$EQS_{bioavailable}$ values for nickel and lead (measured annual average metal concentration > AA- $EQS_{bioavailable}$). ARSO provided us with this data, which we then analyzed in the second step.

Step 2: Verification of the compliance of bioavailable metal concentrations

All inconsistent annual averages of measured concentrations of nickel in the water were recalculated using the bio-met bioavailability tool, and thus we obtained calculated annual averages of bioavailable concentrations. For lead, equations (1), (2), and (3) were used along with the bio-met bioavailability tool, which facilitated a comparison of results from the two different models. The lead concentration can only be calculated with the bio-met model in the model's latest version (5.1, 2022), and we were interested in the difference between the two calculations. This would allow us to decide which of the models for lead is better.

For seawater, neither models nor equations for evaluating bioavailable concentrations have been developed. Therefore, to assess the compliance of sea monitoring data, measured dissolved concentrations are used that, in accordance with the instructions of the WFD, are compared with AA-EQS and MAC-EQS, without considering bioavailability.

3.1 Analysis of annual average concentrations of nickel in water and compliance with AA-$EQS_{bioavailable}$

In the first step, ARSO checked the database of inland surface water monitoring data for nickel during the period 2018-2021 (ARSO, 2022) for the compliance of the annual averages of nickel measurements with AA-$EQS_{bioavailable}$. The bioavailable AA-EQS for nickel is determined in the EQS directive (Council of the European Union, 2013) and amounts to 4 µg/L. ARSO then provided us with data where the EQS value was exceeded.

We found out that 7 of the measured annual average concentrations of dissolved nickel that exceeded the $EQS_{bioavailable}$ value of 4 µg/L belonging to 3 sampling sites (Table 1). The exceeded values of nickel were measured in all four studied years, namely in 2018, 2019, 2020, and 2021.

In step 2, we used the bio-met bioavailability tool v.5.1 to calculate the annual average of bioavailable nickel concentrations. 7 annual average concentrations of dissolved nickel from 3 measuring sites that exceeded the $EQS_{bioavailable}$ value were input into the bio-met model, and the annual average bioavailable concentrations of nickel were calculated (Table 1).

Table 1 shows that the annual average bioavailable nickel concentrations in the water are lower than the measured dissolved nickel concentrations, but that an AA-$EQS_{bioavailable}$ of 4 µg/L is still exceeded for all measurements. The bioavailable concentrations of nickel range between 25.3% (Podvin iztok) and 100% (Meža za tovarno TAB Žerjav), according to the measured concentrations of total dissolved nickel. We can see that, at the measuring site where nickel is 100% bioavailable, the DOC values and calcium ion concentrations are lower than at the other measuring sites and are 1.7 mg/L and 33.1 mg/L, respectively. We estimate that this water is "sensitive" and that nickel is highly bioavailable here due to the low concentrations of DOC and calcium ions in the water. Because bioavailable concentrations of nickel are not compliant with AA-$EQS_{bioavailable}$, for further evaluation, the BLM can be used to calculate the bioavailable concentration of nickel according to the guidelines (European Commission, 2019) in step 3 of the compliance assessment, or local background concentrations of nickel can be considered.

3.2 Analysis of annual average lead concentrations in water, and compliance with AA-$EQS_{bioavailable}$

In the 1st step, ARSO checked for the compliance of the annual averages of lead measurements with AA-$EQS_{bioavailable}$ in the database of inland surface water monitoring data for lead during the period 2018-2021 (ARSO, 2022). The bioavailable AA-EQS for lead is determined in the EQS Directive (Council of the European Union, 2013) and amounts to 1.2 µg/L. ARSO then provided us with data where the EQS value was exceeded.

We found that 35 of the annual average concentrations of dissolved lead in water at 16 measurement sites exceeded the AA-$EQS_{bioavailable}$ value for lead. AA-$EQS_{bioavailable}$ values were exceeded in 2018, 2019, 2020, and 2021.

In step 2, we used two methods to calculate the annual average of bioavailable lead concentrations, namely the bio-met bioavailability tool v.5.1 and equations (1), (2), and (3). We input the annual average concentrations of dissolved lead from the 16 measuring sites that exceeded AA-$EQS_{bioavailable}$ into the bio-met model and calculated annual average bioavailable concentrations of lead using the bio-met model and using the equations (Table 2).

Table 1: *Annual averages of measured and bioavailable concentrations of nickel for measuring sites where the AA-$EQS_{bioavailable}$ for nickel is exceeded.*

Preglednica 1: *Letna povprečja izmerjenih in biorazpoložljivih koncentracij niklja za merilna mesta, kjer je AA-$OSK_{biorazpoložljiv}$ presežena.*

Sample Name	Year	pH	DOC	Ca	Ni_{meas}	Ni_{bioav}
Unit			mg/L	mg/L	µg/L	µg/L
IŠČICA Ižanska cesta	2021	8.0	2.8	59.4	7.8	4.3
PODVIN iztok	2021	7.9	8.4	78.5	167.9	49.2
MEŽA za tovarno TAB Žerjav	2020	8.2	1.7	33.1	41.1	41.1
PODVIN iztok	2020	7.8	9.0	78.0	66.9	16.9
IŠČICA Ižanska cesta	2019	8.1	2.5	54.0	5.6	4.4
PODVIN iztok	2019	7.9	6.7	74.5	56.0	19.2
IŠČICA Ižanska cesta	2018	8.0	2.6	61.5	8.5	5.0

Ni_{meas} – the annual average measured concentration of nickel

Ni_{bioav} – the annual average bioavailable concentration of nickel (bio-met model v.5.1)

Table 2: *Annual averages of measured and bioavailable concentrations of lead for measuring sites where the AA-$EQS_{bioavailable}$ for lead is exceeded.*

Preglednica 2: *Letna povprečja izmerjenih in biorazpoložljivih koncentracij svinca za merilna mesta, kjer je AA-$OSK_{biorazpoložljiv}$ presežena.*

Sample Name	Year	pH	DOC	Ca	Pb_{meas}	$Pb_{bioav\text{-}bio\text{-}met}$	$Pb_{bioav\text{-}equat}$
Unit			mg/L	mg/L	µg/L	µg/L	µg/L
MEŽA za tovarno TAB Črna	2020	8.3	0.9	26.3	2.8	1.50	3.05
MEŽA za tovarno TAB Žerjav	2020	8.2	1.7	33.1	92.4	28.09	56.03
MEŽA pred tovarno TAB Žerjav 1	2020	8.3	1.7	30.6	4.8	1.45	2.86
MEŽA Polena	2020	8.4	1.3	40.7	6.1	2.24	4.69

MEŽA Mežica	2020	8.3	1.6	44.3	4.9	1.56	3.14
HUDI GRABEN Žerjav	2020	8.5	1.0	48.0	4.2	2.05	4.38
MEŽA za tovarno TAB Črna	2019	8.3	1.0	28.0	5.5	2.67	5.66
MEŽA za tovarno TAB Žerjav	2019	8.3	1.5	36.5	12.5	4.18	8.63
MEŽA pred tovarno TAB Žerjav 1	2019	8.2	1.5	31.5	5.1	1.64	3.42
MEŽA pred Hudim Grebenom	2019	8.3	1.2	37.0	15.7	6.30	13.06
MEŽA Mežica	2019	8.4	1.5	44.0	11.5	3.85	7.96
MEŽA pred tovarno Lek – Prevalje	2019	8.3	1.3	38.0	7.8	2.85	5.97
MEŽA pred ind. cono Ravne	2019	8.2	1.2	35.0	2.4	0.96	2.00
MEŽA za ind. cono Ravne	2019	8.2	1.4	35.0	2.2	0.76	1.58
MEŽA Podklanc	2019	8.3	1.4	36.7	1.8	0.66	1.36
HELENSKI POTOK Črna	2019	8.3	2.1	57.0	53.9	13.24	25.68
MUŠENIK Mušenik	2019	8.2	1.8	52.0	4.8	1.29	2.69
PRITOK MEŽE Mušenik	2019	8.5	1.1	57.0	15.9	7.80	14.43
JAZBINSKI POTOK Žerjav	2019	8.3	1.4	47.0	4.0	1.36	2.83
JUNČARJEV POTOK Breg	2019	8.4	2.1	48.0	33.7	7.58	16.06
MEŽA za tovarno TAB Črna	2018	8.2	1.0	26.3	14.0	6.77	14.47
MEŽA za tovarno TAB Žerjav	2018	8.3	1.6	29.5	17.8	5.40	11.51
MEŽA pred tovarno TAB Žerjav	2018	8.3	1.5	30.7	8.3	2.63	5.69
MEŽA pred tovarno TAB Žerjav 1	2018	8.1	2.0	30.0	11.3	2.66	5.80
MEŽA pred Hudim Grebenom	2018	8.2	1.5	42.3	11.6	3.68	7.93
MEŽA Mežica	2018	8.1	1.4	42.3	13.0	4.34	9.07

MEŽA pred tovarno Lek – Prevalje	2018	8.2	1.2	38.3	5.4	2.08	4.38
MEŽA pred ind. cono Ravne	2018	8.2	1.4	37.7	3.7	1.28	2.67
MEŽA za ind. cono Ravne	2018	8.2	1.6	38.7	2.7	0.87	1.75
MEŽA Podklanc	2018	8.3	1.7	39.8	2.2	0.68	1.36
HELENSKI POTOK Črna	2018	8.3	2.2	59.0	64.6	15.86	29.83
MUŠENIK Mušenik	2018	8.3	2.0	55.0	4.6	1.09	2.36
PRITOK MEŽE Mušenik	2018	8.3	1.1	59.0	15.2	7.47	13.41
JAZBINSKI POTOK Žerjav	2018	8.3	1.4	48.0	5.3	1.81	3.89
JUNČARJEV POTOK Breg	2018	8.3	2.3	49.7	32.5	6.71	14.14

Pb_{meas} – the annual average measured concentration of lead

$Pb_{bioav\text{-}bio\text{-}met}$ – the annual average bioavailable concentration of lead (bio-met model v.5.1)

$Pb_{bioav\text{-}equat}$ – the annual average bioavailable concentration of lead (equations (1), (2), and (3))

Table 2 shows that the annual average bioavailable lead concentrations in the water calculated both with the bio-met model ($Pb_{bioav\text{-}bio\text{-}met}$) and with equations (1), (2), and (3) ($Pb_{bioav\text{-}equat}$) are lower than the measured dissolved concentrations. It can also be observed that the $Pb_{bioav\text{-}bio\text{-}met}$ concentrations are 2 times lower than $Pb_{bioav\text{-}equat}$ concentrations. Specifically, that AA-$EQS_{bioavailable}$ (1.2 µg/L) is exceeded in all cases when using $Pb_{bioav\text{-}equat}$ calculations, while with $Pb_{bioav\text{-}bio\text{-}met}$ calculations, 6 measurements at 4 measurement sites do not exceed the AA-$EQS_{bioavailable}$.

We believe that this difference occurs because the bio-met model considers, in addition to the DOC concentration, the concentration of calcium ions and the pH of the water when calculating the bioavailable concentration. Apart from this, the bio-met calculation is based on experimental ecotoxicological data. We estimate that the use of the bio-met model for calculating the concentration of bioavailable lead is more reliable than the use of equations. In 2017, when bioavailable concentrations started being calculated for compliance assessment, the bio-met model for lead was not yet available. Therefore, we suggest that ARSO considers using the bio-met model to calculate the concentration of bioavailable lead.

The values of bioavailable concentration calculated with bio-met vary between 20.6% (Junčarjev potok Breg) and 53.6% (Meža za tovarno TAB Črna), according to the measured concentrations of total dissolved lead.

For further evaluation of the bioavailable concentrations of lead that are not compliant with AA-$EQS_{bioavailable}$, the BLM model can be used to calculate the bioavailable concentration of lead, according to the guidelines (European Commission, 2019) in step 3 of the compliance assessment, or local background concentrations of lead can be considered.

4. Conclusions

In this paper, the importance of the consideration of bioavailable concentrations of metals in water according to the Water Framework Directive (Council of the European Union, 2000) (WFD) is demonstrated, and the use of the biotic ligand model (BLM) and the user-friendly models (bio-met

bioavailability tool, M-BAT and PNEC-pro) for assessing the bioavailability of metals in water is explained. When calculating the annual average bioavailable concentrations of nickel and lead in water, we suggest utilising the bio-met bioavailability tool model. We also presented a model with three equations for calculating the annual average bioavailable concentration of lead based on data gathered at the location.

Furthermore, we performed an analysis of inland surface water monitoring data for nickel and lead, and checked how the introduction of annual average bioavailable concentrations affects compliance with the annual average environmental quality standards, which refer to biologically available concentrations ($AA\text{-}EQS_{bioavailable}$) for these metals. In doing so, we started from the national monitoring of inland surface water (river and lakes) data from the period 2018-2021. When using bio-met model, the calculation of annual average bioavailable concentrations of nickel and lead is based on data on the concentrations of these metals, of DOC, of calcium ions, and on pH-values. The measured lead concentration and concentration of DOC are used in equations (1), (2), and (3) in calculating the bioavailable concentration of lead.

Of the 7 annual average measured concentrations of nickel from 3 measuring sites that exceed $AA\text{-}EQS_{bioavailable}$, all exceed $AA\text{-}EQS_{bioavailable}$ standards after calculating the annual average biological concentrations but are lower than the measured dissolved concentrations of nickel. The exception is one measurement where nickel is 100% bioavailable due to sensitive water conditions. Following from this, we recommend implementing the 3rd step of determining compliance (European Commission, 2019), namely the use of the local nickel background concentration, and/or the use of the BLM to calculate the bioavailable nickel concentrations.

To calculate the annual average bioavailable concentrations of lead, we used two methods, namely the bio-met bioavailability tool v.5.1 and equations (1), (2), and (3). For equations (1), (2), and (3), we used measured dissolved lead concentrations along with DOC data. For the bio-met bioavailability tool, we incorporated measured dissolved lead concentrations, DOC, calcium ion concentration, and pH data.

It was found that 35 annual average measured concentrations of dissolved lead in water at 16 measurement sites exceeded $AA\text{-}EQS_{bioavailable}$. All annual average bioavailable lead concentrations in water calculated with the bio-met model and equations (1), (2), and (3) are lower than the measured annual dissolved lead concentrations. However, all bioavailable concentrations calculated with the bio-met model are approximately 2 times lower than those calculated with the equations.

We estimate that this is because, while equations (1), (2), and (3) solely consider the concentration of dissolved lead and dissolved organic carbon (DOC), the bio-met bioavailability tool model also takes into consideration the concentration of calcium ions and the pH of the water. These contribute to a more accurate estimate of the biologically available lead concentration.

Apart from this, the bio-met calculation is based on experimental ecotoxicological data. Based on the presented data, we estimate that the use of the bio-met model in calculating the bioavailable lead concentration is more reliable than the use of equations. Therefore, for the annual assessment of the compliance of inland surface water monitoring data carried out by ARSO, we propose that employing the bio-met bioavailability tool model to calculate bioavailable lead concentration is a more dependable approach than that of using the three equations.

Bioavailable concentrations of lead calculated with the bio-met model for 6 measurements at 4 measurement sites no longer exceed the $AA\text{-}EQS_{bioavailable}$ limit. For other non-compliant measurement sites, we recommend the implementation of step 3 of compliance assessment (European Commission, 2019), namely the use of local background lead concentration, and/or the use of the BLM to calculate the concentrations of bioavailable lead.

Our work has driven an upgrade in evaluating environmental standards for metals and their compounds. This has enabled the introduction of the requirements of Directive 2013/39/EC (Council of

the European Union, 2013), which refer to biologically available concentrations of substances ($EQS_{bioavailable}$).

Acknowledgments

This work was funded by the Slovenian Environment Agency. The study was part of the national project on the implementation of the Directive 2008/105/EC and Directive 2013/39/EU into Slovenian legislation (2014).

References

ARSO (2022). Podatki o kakovosti voda. Available at: http://www.arso.gov.si/vode/podatki/ (in Slovenian) (retrieved 15/2/2023).

Bio-met (2017). Bio-met bioavailability tool User Guide (version 5.1). Guidance document on the use of the bio-met bioavailability tool. Available at www.bio-met.net (retrieved 15/2/2023), 48 p.

Council of the European Union, (2008). Directive 2008/105/EC of the European Parliament and of the Council of 16 December 2008 on environmental quality standards in the field of water policy. European Commission and Council.

Council of the European Union (2013). Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy. European Commission and Council.

Council of the European Union (2000). Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. European Commission and Council.

Cousins, A.P., Jönsson, A., Iverfeldt, Å. (2009). Testing the Biotic Ligand Model for Swedish surface water conditions. IVL Swedish Environmental Research Institute, Stockholm, 61 p.

European Chemicals Agency (2008). Guidance on information requirements and chemical safety assessment Chapter R.10. Characterisation of dose [concentration]-response for environment. Helsinki, 65 p.

European Commission (2019). Common Implementation Strategy for the WFD. Guidance Document No. 38. Technical Guidance for implementing Environmental Quality Standards (EQS) for metals. Consideration of metal bioavailability and natural background concentrations in assessing compliance. Brussels, 128 p.

European Commission (2018). Common Implementation Strategy for the WFD. Technical Guidance for Deriving Environmental Quality Standards. Guidance Document No. 27. Updated version 2018. Brussels, 210 p.

Merrington, G. (2015). Technical Guidance to Implement Bioavailability-Based EQS for Metals. WCA Environment, Oxfordshire, 73 p.

Peters, A. (2011). Biotic Ligand Models. Application to Data Poor Metals. WCA Environment, Oxfordshire, 18 p.

Peters, A., Schlekat, C.E., Merrington, G. (2016). Does the scientific underpinning of regulatory tools to estimate bioavailability of nickel in freshwaters matter? The European-wide environmental quality standard for nickel. *Environmental Toxicology and Chemistry* **35 (10**), 2397–2404. https://doi.org/10.1002/etc.3510.

Santore, R.C., di Toro, D.M., Paquin, P.R., Allen, H.E., Meyer, J.S. (2001). Biotic ligand model of the acute toxicity of metals. 2. Application to acute copper toxicity in freshwater fish and Daphnia. *Environmental Toxicology and Chemistry* **20 (10)**, 2397–2402. https://doi.org/10.1002/etc.5620201035.

Sokal, R.R., Rohlf, F.J. (1995). Biometry, Third Edit. W. H. Freeman and Company, New York, 887 p.

The European Parliament and the Council of the EU, (2007). REACH Regulation (EC) No 1907/2006. The European Parliament and the Council of the EU.

United Kingdom and SCHER (2011). Lead and its compounds EQS draft dossier 20110124 – Voluntary European Union Risk Assessment Report. European Commission, 64 p.

van Vlaardingen, P., Traas, T.P., Wintersen, A.M., Aldenberg, T. (2004). ETX 2.0 – A Program to Calculate Hazardous Concentrations and Fraction Affected, Based on Normally Distributed Toxicity Data. RIVM, Bilthoven, 68 p.

Vink, J., Verschoor, A. (2010). Biotic Ligand Models – availability, performance and applicability for water quality assessment. Deltares, Delft, 36 p.