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The threshold concentration for nitrate in groundwater as a habitat of *Proteus anguinus*

Mejne koncentracijske vrednost za nitrat v podzemni vodi kot okolju močerila

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The aim of the study was to assess the risk that nitrate might pose to the groundwater ecosystem in the LIFE Kočevsko project area (http://life-kocevsko.eu). We identified most relevant sources of nitrate in groundwater of the project area as well as in the entire karst region where the proteus (*Proteus anguinus*) populations are present. Based on toxicity data on amphibians we calculated the threshold concentration for nitrate in groundwater as a habitat of proteus. The main sources of nitrate emissions in groundwater were identified as wastewater treatment plant effluents that immediately sink into karst underground, as well as emissions from livestock farming and the potentially inappropriate use of manure. The calculated threshold concentration of nitrate for proteus of 9.2 mgNO_3^-/L comprises the predicted no-effect concentration (PNEC), the natural background concentration and the expected variation of the natural background concentration. Based on results obtained, we proposed possible risk mitigation measures to reduce the impact of nitrate on groundwater as the proteus' habitat.

The groundwater directive provides the groundwater quality standard (GQS) for nitrate of 50 mgNO_3^-/L (2006/118/EC 2006). This value is based on epidemiological evidence for methaemoglobinaemia in infants, which results from short-term exposure to nitrate. The nitrate GQS is protective for bottle-fed infants and, consequently, other population groups (World Health Organization 2011). It is obvious that the goal of nitrate GQS is to protect groundwater as a source of drinking water and not as an ecosystem. However, several scientific publications provide information that this value might not be safe for the aquatic ecosystems. It seems that amphibians are more sensitive in their developmental stages than humans (Marco et al. 1999, Rouse et al. 1999). The presented survey was focused on the LIFE Kočevsko project area in southern Slovenia, mainly in the Municipality of Kočevje. However, a broader view on the potential emission of nitrate in the proteus' habitats in the karst region of Slovenia was also presented. Two main sources of nitrate emissions in groundwater were identified:

- Wastewater treatment plants (WWTP) effluents that immediately sink into the karst underground.
- Emissions from livestock farming and the potentially inappropriate use of manure.

Wastewater treatment effluents in the karst region commonly sink directly into the underground and groundwater. The effluents from the tertiary WWTP are, in most cases, of a good quality (regarding the organic pollution, nitrates and phosphorous). However, the secondary treatment, as the most common method of treatment in smaller WWTP, does not provide the adequate effluent quality. Such an example is the WWTP on the border of the project area near Ribnica na Dolenjskem, which releases effluents into the sinker and groundwater.

Intensive pig and cattle production estates are located within the project area. In addition, spreading of manure from the intensive poultry production over the grassland might exert a strong influence on the groundwater habitats of the proteus populations.

The threshold concentration of nitrate for proteus comprises the predicted no-effect concentration (PNEC), the natural background concentration and the expected variation of the natural background concentration. The PNEC is extracted from selected long-term toxicity data (expressed as NOAEL – no observed adverse effect level or NOEC – no observed effect concentration) of $NaNO_3$ and KNO_3 on amphibians available in scientific literature (e.g. Schuytema & Nebeker 1999a, Laposata & Dunson 1998, Camargo et al. 2005).

The predicted no-effect concentration (PNEC) calculation was performed following the method using SSD (Species Sensitivity Distribution) approach and the ETX 2.1. software (van Vlaardingen et al. 2014): the PNEC was calculated based on the 5th percentile (HC5) of toxicity data (Tabs. 1, 2; Fig. 1) by applying the assessment factor 1.

Table 1. Overview of endpoints of toxicity expressed with NOAEL or NOEC, for different amphibians, taken from published references. Markings refer to: * – embryo, ** – tadpole.
Tabela 1. Vrednosti parametrov toksičnosti NOAEL ali NOEC za različne dvoživke, vzete iz literature. Oznake se nanašajo na: * – zarodek, ** – paglavec.

Number	NOAEL/NOEC [mg/L]	Tested species	Reference
1	9.0	*Ambystoma maculatum**	Laposata & Dunson 1998
2	9.0	*Ambystoma jeffersonianum**	Laposata & Dunson 1998
3	9.0	*Rana sylvatica***	Laposata & Dunson 1998
4	66.0	*Xenopus laevis***	Schuytema & Nebeker 1999b, Sullivan & Spence 2003
5	56.7	*Pseudacris regilla**	Schuytema & Nebeker 1999a
6	78.2	*Pseudacris regilla*** – average values of 30.1^{A} and 126.3^{B}	A – Schuytema & Nebeker 1999b, B – Camargo et al. 2005
7	29.0	*Rana aurora**	Schuytema & Nebeker 1999c
8	24.8	*Xenopus laevis**	Schuytema & Nebeker 1999a
9	5	*Rana temporaria***	Johansson et al. 2001
10	9	*Bufo bufo***	Baker & Waights 1993

Table 2. Values of the SSD calculation (software ETX 2.1., van Vlaardingen et al. 2014) is expressed as the 5^{th} percentile (HC5) of the long-term effect concentrations of $NaNO_3$ on embryonal and/or larval stage of amphibians.
Tabela 2. Izračun SSD (programsko orodje ETX 2.1., van Vlaardingen et al. 2014) temelji na vrednosti 5 percentile (HC5) koncentracij dolgodobnih učinkov $NaNO_3$ na embrionalne in/ali larvalne stadije dvoživk.

Name	Value	Log10 (value)	Description
LL HC5	1.04	0.019	lower estimate of the HC5
HC5	**3.50**	**0.544**	**median estimate of the HC5**
UL HC5	6.95	0.842	upper estimate of the HC5
sprHC5	6.65	0.823	spread of the HC5 estimate

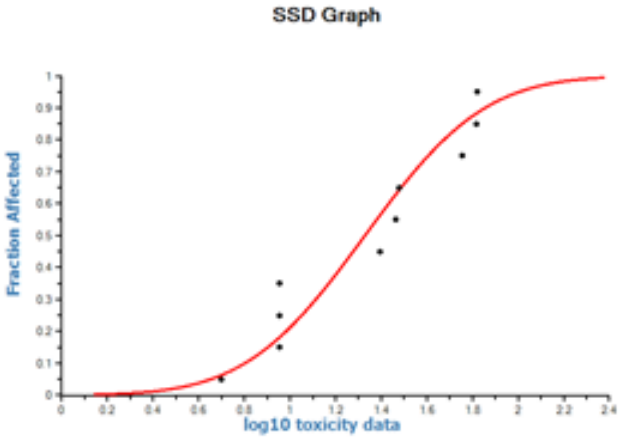

Figure 1. Graphic display of the species sensitivity distribution (SSD) (software ETX 2.1., van Vlaardingen et al. 2014).
Slika 1. Grafični prikaz porazdelitve občutljivosti vrst (SSD – species sensitivity distribution) (programsko orodje ETX 2.1., van Vlaardingen et al. 2014).

The natural background concentration for nitrate in Slovenia is 3.8 $mgNO_3^-/L$ (Mezga 2014). This concentration is estimated for all the areas with identified proteus populations. Therefore, the calculated threshold concertation for nitrate in groundwater can be applied to all these sites. The deviation of the natural background concentration of nitrate was estimated to be 50% (1.9 $mgNO_3^-/L$).

Calculation of PNEC:
$PNEC_{SSD}$= HC5/AF
$PNEC_{SSD}$=3.5 mg NO_3^-/L
The expected background concentration of nitrate: 3.8 $mgNO_3^-$/L
50% of expected deviation of the natural background concentration: 1.9 $mgNO^-_3$/L
The proposed threshold concentration for proteus: 9.2 $mgNO_3^-$/L

The proteus is one of the most remarkable representatives of stygofauna in Slovenia and in Europe. Emissions from agriculture and wastewater effluents can pose a threat to existing populations of this neotenic amphibian. To reduce the risk of nitrate to the proteus, we propose several risk mitigation measures that the risk manager should apply in the LIFE Kočevsko project area as well as at other exposed locations in the karst regions. The measures are as follows:

- To implement the threshold value of 9.2 $mgNO3^-$/L in groundwater as an environmental quality standard for good chemical status for the proteus habitats.
- To implement appropriate measures within subvention policy to enhance good agricultural practice of manure use and penalize the pollution of environmental compartments with manure.
- To introduce a strict recording of manure application on farms in the karst region.
- Surveillance over the adequacy of dung pits, dung collection sites and possible leaks of slurry into the environment.
- A network of stakeholders, NGOs and public bodies that might have an interest should be established and invited to identify and record all possible sources of nitrates in groundwater.
- Implementation of legal terms that would prevent release of untreated or insufficiently treated wastewater to sink directly into the karst underground and groundwater.

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