Preliminary Ecological Risk Assessment to Aquatic Environment of Bega River Due to Presence of Nitrates in Treated Sewage Effluent

Marius Gheju[*], Corneliu Bogatu[**], Dimitrie Botau[**], Elena Savescu[***], Dalila Marsavina[***], Mihaela Puiulet[***]

* "Politehnica" University of Timisoara, Faculty of Industrial Chemistry and Environmental Engineering, Bd. V. Parvan Nr. 6, Et. 4, 1900, Timisoara, Romania, e-mail: marius.gheju@chim.upt.ro

** National R&D Institute for Industrial Ecology – ECOIND, PO Box 254 Of.1, P-ta Victoriei Nr.2, Et.2, 1900 Timisoara, Romania, e-mail: corneliu.bogatu@rectorat.utt.ro

***Water and Wastewater Treatment Company, AQUATIM Timsoara, Strada Gheorghe Lazar nr.11 A, tel. +40 256 201370, e-mail: dalila.marsavina@aquatim.ro

Abstract: This paper presents the occurrence of nitrate ions in effluent from Timisoara sewage treatment plant and the assessment of risk it may pose to aquatic environment of Bega River. An average concentration of 13.33 mg NO_3^-/l was reported in Timisoara sewage treatment plant effluent. The risk assessment to aquatic environment due to presence of nitrate in treated sewage was evaluated according to the procedure laid down in European Union. Because a RQ < 1 was obtained, this indicates that nitrates ions from effluent of Timisoara sewage treatment plant do not pose any risk to the aquatic environment of Bega River.

Keywords: Risk assessment, nitrate toxicity, aquatic environment, surface waters, sewage treatment.

1. Introduction

Oxides of nitrogen, such as nitrate and nitrite, are common pollutants in surface water systems and ground water contaminated with nitrogenous compounds. Increasing concentrations of nitrate in surface water and groundwater are becoming a worldwide concern [1]. The concentration of nitrates in unaffected rivers should be 0.1 - 0.5 mg/l. In Europe, the highest concentrations were found in the intensive agricultural regions in the northern part of Western Europe, where 68% of the rivers have mean nitrate concentrations exceeding 1 mg/l. In the Nordic countries nitrate concentrations are low, 70% of the rivers have levels below 0.3 mg/l. The most important anthropogenic sources of nitrate load in surface waters are agricultural activities, animal farming, and effluents from sewage treatment plants that are not performing tertiary treatments [2,3]. Therefore, it is possible that nitrate discharges from anthropogenic sources may result in a ecological risk for certain aquatic species.

Timisoara sewage treatment plant was build between 1909 and 1912. At that time, the plant had a total installed capacity of 570 l/s, and urban sewage was treated exclusively by mechanical processes. In our days, the sewage treatment plant has an installed capacity for the mechanical treatment and for the biological treatment of 3500 l/s and 2000 l/s, respectively. The sewage treatment process consists in primary treatment and secondary treatment. Primary treatment consists of the removal of suspended solids and insoluble matter from water, such as: large floating objects, grit, scum, oils and greases; this treatment phase involves the following physical unit operations: bar screens, grit separation chamber, grease separation chamber and primary clarifier basin. Secondary treatment is designed to reduce biological oxygen demand (BOD), by using biological processes; this treatment phase consists of the following unit operations: activated sludge aeration basin, and secondary clarifier basin. A portion of the activated sludge that settle in the secondary clarifier is circulated back to the aeration basin, and the remaining sludge is further processed. At this time, a tertiary sewage treatment unit, for the removal of nitrogen and phosphorus, is under construction. The treated effluent was discharged in 2006 with a main flow rate of 135,744 m^3/day into the Bega River.

This study's research goal was to assess the risk to aquatic environment of Bega River, posed by the presence of nitrates in effluent from Timisoara sewage treatment plant.

2. Methodology

Ecological risk assessment is a process for evaluating the likelihood that adverse ecological effects may occur or are occurring as a result of exposure to one or more stressors [4]. There are two main procedures that can be used for the ecological risk assessment, both based on effect assessment and exposure assessment.

According to the Guidelines for Ecological Risk Assessment (GERA), a procedure laid down in USA, ecological risk assessment is a process comprised of several steps: (1) problem formulation, (2) analysis, and (3) risk characterization. First phase is a process for generating and evaluating hypotheses about why ecological effects have occurred, or may occur, from human activities. Problem formulation results in three products: assessment endpoints, conceptual models, and an analysis plan. The analysis phase includes two principal activities:

characterization of exposure and characterization of ecological effects. Exposure characterization describes sources of stressors, their distribution in the environment, and their contact or co-occurrence with ecological receptors. An ecological effect characterization evaluates stressor - response relationships or evidence that exposure to stressors causes an observed response. Risk characterization is the final phase of an ecological risk assessment, and consists in the estimation of ecological risks through integration of exposure and stressor-response profiles [5].

According to the Technical Guidance Document on Risk Assessment (TGDRA), a procedure laid down in European Union, the ecological risk assessment process consists in three main actions: (1) assessment of effects, (2) exposure assessment, and (3) risk characterization. First phase includes two principal activities: hazard identification and dose (concentration) - response (effects) assessment. Hazard identification is a process for identification of the adverse effects, which a substance has an inherent capacity to cause. Dose - response assessment estimate the relationship between dose, or level of exposure to a substance, and the incidence and severity of an effect, where appropriate. The second phase estimate the concentrations/doses to which environmental compartments (aquatic environment, terrestrial environment and air) are or may be exposed. The final phase estimate the incidence and severity of the adverse effects likely to occur in a environmental compartment due to actual or predicted exposure to a substance [6].

In this study, the risk assessment to aquatic environment due to presence of nitrate in treated sewage was evaluated according to the procedure laid down in European Union.

2.1. Effects assessment

The assessment of effects must answer to the following question: what potential adverse effects might the contaminant of concern cause and at what concentration? This phase comprises the following steps of the risk assessment procedure: (1) hazard identification, and (2) dose (concentration) - response (effect) assessment [6].

2.1.1. Hazard identification

This phase must identify the effects of concern resulted from aquatic environment exposure to nitrates. The main toxic action of nitrate on aquatic animals, but also on human health, is due to the conversion of nitrate to nitrite in the body. The major effect of nitrite is that it reduces the capacity of the blood to transport oxygen due to the conversion of oxygen-carrying agents (e.g. hemoglobin) to forms that are incapable of carrying oxygen. (e.g. methemoglobin) [7,8]; in addition, nitrite reacts with compounds in the stomach to form products that have been found to be carcinogenic in many animal species, although the link to cancer in humans is at the moment suggestive.

Nitrate toxicity to aquatic animals increases with increasing nitrate concentrations and exposure times. In contrast, nitrate toxicity may decrease with increasing body size, water salinity, and environmental adaptation. Freshwater animals appear to be more sensitive to nitrate than marine animals [9].

2.1.2. Concentration - effect assessment

The aim of this phase is to estimate the relationship between levels of exposure to nitrate and severity of observed effects. At this step the predicted no effect concentration of nitrite in water (PNEC) will also be determined. The PNEC is the concentration below which an unacceptable effect will most likely not occur. For the aquatic environment, a PNEC is derived that, if not exceeded, ensures an overall protection of the environment. Some assumptions are made concerning the aquatic environment which allow an extrapolation to be made from single-species short-term toxicity data to ecosystem effects. It is assumed that: (1) ecosystem sensitivity depends on the most sensitive species, and (2) protecting ecosystem structure protects community function [6].

As ecological endpoints were chosen three species which may be found in the aquatic environment of Bega River: aquatic invertebrates (*Daphnia magna* and *Ceriodaphnia dubia*), fishes (*Pimephales promelas*) and amphibians (*Rana temporaria*).

Daphnia magna and *Ceriodaphnia dubia* are small, mostly planktonic, crustaceans. Daphnia are one of the several small aquatic crustaceans commonly called water fleas because of their saltatory swimming style. They live in various aquatic environments ranging from acidic swamps to freshwater lakes, ponds, streams and rivers. The scientific classification of *Daphnia magna* and *Ceriodaphnia dubia* is presented in Tables 1 and 2, respectively [10]. The acute and chronic toxicity of nitrate (NO_3-N) to *D. magna* and *C. dubia* was investigated by Scott et al. [7] in a 48 hours to 17 days exposure tests. The NOEC (no-observed effect concentration) and LOEC (lowest-observed-effect concentration) values for neonate production in *D. magna* were 358 and 717 mg/L NO_3-N, respectively. The NOEC and the LOEC for neonate production in *C. dubia* were 21.3 and 42.6 mg/L NO_3-N, respectively. The 48-h median lethal concentration (LC50) of nitrate to *C. dubia* and *D. magna* neonates was 374 mg/L NO_3-N and 462 mg/L NO_3-N, respectively.

Pimephales promelas, also known as the fathead minnow, is a stream fish, able to tolerate a wide range of environmental conditions including high temperatures, low oxygen levels, and high turbidities. The species can be found in muddy ponds and streams that might otherwise be inhospitable to others species of fish. The scientific classification of *P. promelas* is presented in Table 3 [10]. The acute and chronic toxicity of nitrate (NO_3-N) to *P. promelas* was investigated by Scott et al. [7] in a 48 hours to 17 days exposure tests. The 96-h median lethal concentration LC50 for larval *P. promelas* was 1.341 mg/L NO_3-N. The NOEC and LOEC for 7 days larval and 11

days embryo–larval growth tests were 358 and 717 mg/L NO_3-N, respectively. *Rana temporaria*, also known as the European Common Frog or European Common Brown Frog, is found throughout much of Europe as far north as the Arctic Circle and as far east as the Urals. The scientific classification of *R. temporaria* is presented in Table 4 [10]. The acute and chronic toxicity of nitrate (NO_3-N) to *R. temporaria* was investigated by Johansson et al. [11] with the aim to test whether common larvae from northern parts of Scandinavia are less well adapted to cope with high nitrate concentrations than those from the southern parts. Authors reported that high nitrate concentrations reduced the growth rates and metamorphic size in north, but not in south; significant mortality occurred after 24 h exposure in the two highest concentrations (0.5–1 mg/l), and 72 h exposure reduced survival to near zero also in 0.1 mg/l.

TABLE 1. Scientific classification of Daphnia magna.

Kingdom	Animalia
Phyllum	Arthropoda
Subphyllum	Crustacea
Class	Branchiopoda
Order	Cladocera
Family	Daphniidae
Genus	Daphnia

TABLE 2. Scientific classification of Ceriodaphnia dubia.

Kingdom	Animalia
Phyllum	Arthropoda
Subphyllum	Crustacea
Class	Branchiopoda
Order	Cladocera
Family	Daphniidae
Genus	Ceriodaphnia

TABLE 3. Scientific classification of Pimephales promelas.

Kingdom	Animalia
Phyllum	Chordata
Class	Actinopterygii
Order	Cypriniformes
Family	Cyprinidae
Genus	Pimephales
Species	Pimephales promelas

TABLE 4. Scientific classification of Rana temporaria.

Kingdom	Animalia
Phylum	Chordata
Class	Amphibia
Order	Anura
Suborder	Neobatrachia
Family	Ranidae
Genus	Rana
Species	Rana temporaria

The PNEC can be calculated by dividing the lowest long-term NOEC value by an appropriate assessment factor. The NOEC is defined as the highest concentration tested at which the measured parameter shows no significant inhibition. According to the TGDRA, an assessment (dilution) factor of 10 can be applied when long-term toxicity NOECs are available from at least three species [6]. The NOECs and the calculated PNEC for the three species mentioned in this study, are presented in Tables 5 and 6, respectively.

TABLE 5. Comparative aquatic toxicity of nitrate-nitrogen to aquatic species.

Species	Developmental stage	NOEC mg NO_3^-- N /l	References
Daphnia magna	Neonates (< 48 h)	358	7
Ceriodaphnia dubia	Neonates (< 24 h)	7.1 - 56.5	7
Pimephales promelas	Larvae (< 24 h)	358	7
Rana temporaria	Larvae	5.0	11

TABLE 6. Predicted no effect concentration (PNEC) of nitrate-nitrogen to aquatic species.

Species	Developmental stage	PNECwater mg NO_3^-- N /l
Daphnia magna	Neonates (< 48 h)	35.8
Ceriodaphnia dubia	Neonates (< 24 h)	0.71 - 5.65
Pimephales promelas	Larvae (< 24 h)	35.8
Rana temporaria	Larvae	0.50

2.2. Exposure assessment

Aquatic biotas are most likely to be exposed to contaminants through direct contact with water or through ingestion of surface water, sediment, and contaminated food. In aquatic systems, organisms are exposed to concentrations of contaminants. This is why the aim of our exposure assessment was to estimate the nitrate concentration to which aquatic biota of Bega River is exposed due to urban treated sewage discharge in this river. As a result of a study conducted between 2 - 4 Jul. 2006 [12], the average NO_3^- concentration in the sewage treatment plant effluent was found to be 13.33 mg NO_3^-/l, as presented in Table 7.

According to the TGDRA, the nitrate concentration in Bega River was estimated after complete mixing of the effluent outfall, considering that dilution was the dominant process. Therefore, nitrate degradation, sedimentation or volatilisation from the water body were not taken into account as possible nitrate removal processes. In order to calculate the predicted environmental nitrate concentration (PEC) in receiving water, TGDRA has suggested a standard dilution factor of 10 [6]. As a result, the predicted environmental nitrate concentration in Bega River is 1.33 mg NO_3^- /l, or 0.30 mg NO_3^-- N /l.

TABLE 7. *Nitrate concentrations in Timisoara sewage treatment plant effluent.*

Date	Time	Nitrate concentration mg NO_3^- /l
02.07.2006	2^{00}	6.4
	8^{00}	22.0
	14^{00}	15.0
	20^{00}	7.4
03.07.2006	2^{00}	1.1
	8^{00}	2.3
	14^{00}	6.5
	20^{00}	23.4
04.07.2006	2^{00}	17.2
	8^{00}	26.5
	14^{00}	28.7
	20^{00}	3.5
Mean		13.33 ± 10.00

2.3. Risk characterization

After conducting the effect assessment and the exposure assessment for the aquatic biota of Bega River, either a quantitative risk characterisation or a qualitative (for cases where a quantitative risk characterisation cannot be carried out) risk characterisation must be carried out. There is a range of possible methods, of variable complexity, for risk characterization. The risk quotient (RQ) is the most widespread method for the quantitative characterization of risks, which consist in direct comparison of the PEC and PNEC values. If the PEC/PNEC ratio is greater than one the substance is "of concern" and further action has to be taken [6].

The risk characterisation to aquatic environment of Bega River, due to presence of nitrate in treated sewage, was carried out by comparing predicted nitrate environmental concentration (PEC) with the predicted nitrate no effect concentration (PNEC) for the most sensitive species to nitrates, *Rana temporaria*. The value of RQ was calculated using equation 1.

$$RQ = \frac{PEC}{PNEC} = \frac{0.30}{0.50} = 0.60 \qquad (1)$$

3. Conclusions

Because RQ < 1, this indicates that nitrates ions from effluent of Timisoara sewage treatment plant are not supposed to pose any risk to the aquatic environment of Bega River. Although this is a favorable conclusion, we believe that there is need for further testing of nitrate concentration in treated sewage effluent, over a longer period of time, in order to obtain a more representative monitoring data, and finally, a more reliable value for the PEC value.

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