EDITORIAL

Microbial source tracking analyses confirmed that the pollution

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ABSTRACT

Atmospheric nitrate deposition or nitrate-containing fertilizers were found not to be significant sources of riverine nitrate in the SRB. The ranges of isotope values suggest that NO3− in the SRB derives from soil nitrification, sewage, and/or manure, which were further supported by MST analysis. Microbiological indicators show the existence of hotspots of fecal pollution in the SRB, which are human associated. Long-term observations indicate persistent fecal contamination at selected locations caused by continuous discharge of untreated wastewaters into the SRB.

INTRODUCTION

The much effort has been invested into understanding the nutrient cycles, heightened by problems associated with global, regional and local environmental problems. These problems result in part from increased fluxes of nutrients such as nitrogen (N) and phosphorous (P) compounds into water bodies that can lead to eutrophication, causing ecological changes including loss of plant and animal species. In addition, these fluxes can influence the quality of water for human consumption and other purposes. In many river catchments the main source of N pollution is run-off from agricultural land, although discharges from wastewater treatment works can also be significant. According to the European Environmental Agency current concentrations of nitrate are still above what might be considered to be 'background' or natural levels, except in the northern European countries. Thus control of these nutrient discharges is needed to reduce pollution levels in water bodies. Isotope techniques constitute a promising tool for determining sources of nitrate (NO_3^-) in surface water and could help to identify the processes that NO_3^- has undergone in the aquatic system. This is possible because the isotopic signature in NO_3^- from fertilizer, atmospheric, soil and manure-derived sources differs sufficiently to enable an unambiguous distinction to be made between them. The contamination of water bodies by sewage or manure is generally determined using the detection of Fecal Indicator Bacteria. Specific differentiation between sources of fecal contamination is of particular importance, because the risk to humans is usually considered higher from human fecal contamination (sewage) than from animal fecal contamination. New approaches based on PCR quantification and biomarker analysis could be used to track microbiological sources of contamination. Land use of the SRB reflects the differences in relief, climate and stream flow. The greatest population density is located near large cities. Three capitals are located near the Sava: Ljubljana, Zagreb and Belgrade. Agriculture is the dominant activity in the Croatian (40%) and Serbian (~100%) parts of the watershed, while the upper part, in Slovenia, is mainly covered by forest (>50%). The Bosnian part of the watershed is dominated by valleys and hills, with about 30% agricultural area and 30% forest. For these four countries, the basin catchment area comprises 60%-70% of their land and is the source of >80% of total available water. Due to important hydropower generation, industrial and agricultural activities, and a high population density, especially near large cities (Ljubljana, Zagreb, and Belgrade) strong anthropogenic influences on water quality are expected.

CONCLUSION

The combined use of the isotope compositions of N and O in NO_3^- provides evidence that NO_3^- is affected primarily by two different sources: one source originating from pristine soils, and another originating from wastewater and manure, although processes such as phytoplankton assimilation and nitrification have also been observed, especially in autumn 2015. According to the analyses of the $\delta^{15}N_{NO3}$ and $\delta^{15}N_{TPN}$ values the minor and/or zero uptake of local nitrate was detected in most locations. According to the $\delta^{18}O_{NO3}$ values it is possible to conclude that atmospheric deposition does not contribute significantly to river NO_3^- nor do the nitrates originating from commercial fertilizers. We therefore suggest that the comparatively low isotopic enrichment factors for nitrogen and oxygen arise from a combination of soil nitrification and an admixture of NO_3^- from wastewater and manure. This assumption could be further confirmed by microbiological analyses. These analyses comprised analysis of the standard indicators of fecal pollution and Microbial Source Tracking (MST) analyses based on the human-associated BacHum and HF183II, the ruminant-associated BacR and the pig-associated Pig2Bac genetic Bacteroidetes fecal markers. The results reveal the existence of hotspots of fecal pollution of human associated origin in the SRB. Long-term data at selected locations indicated persistent fecal contamination, showing that the locations are impacted by continuous discharge of untreated wastewaters. This study describes the present nutrient and microbiological pollution status in the SRB, and indicates the urgent need for effective wastewater treatment plants in water management.

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