



## The impact of gypsum mine water: A case study on morphology and DNA integrity in the freshwater invertebrate, *Gammarus balcanicus*



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### ABSTRACT

The aim of our study was to investigate how exposure to heavy metal-rich waters from gypsum mining affects the morphology and levels of primary DNA damage in *Gammarus balcanicus*. Chemical analysis revealed increased concentrations of metals in water and sediment collected at a site impacted by gypsum mine wastewaters. The specimens also showed elevated total tissue metal levels when compared with the organisms collected at the reference site. The most prominent increase was observed for strontium, followed by iron, nickel, vanadium, aluminium, and manganese. The major pathway of entry for these toxic substances was through the degraded exoskeleton as a consequence of excessive strontium input (unbalanced calcium/strontium ratio) and altered permeability. Disturbed exoskeleton integrity was observed only in individuals collected downstream of the gypsum mine, which was confirmed by electron microscopy. Levels of primary DNA damage were evaluated using the alkaline comet assay in the haemolymph of the specimens.

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### 1. Introduction

Gypsum is a significant ore mineral resource in building, cement production, and a number of technological processes in the chemical industry and elsewhere. Wastewaters resulting from mining activities and especially from gypsum production are contaminated with heavy metals (Tichy et al., 1998; Mihara et al., 2008). These metals can be taken up and accumulated by aquatic organisms, both from the surrounding medium and via food sources. Many of the metals play essential roles in the metabolism, and all have the potential to cause ecotoxicological effects, especially trace metals. One such metal strongly associated with calcareous rocks and gypsum layers is strontium (Warren, 2006). The two most significant strontium minerals are celestite (strontium sulphate; SrSO<sub>4</sub>) and strontianite (strontium carbonate; SrCO<sub>3</sub>).

Strontium compounds can be water soluble; examples include strontium carbonate and strontium chromate. In Croatia, occurrences and deposits of gypsum can be found in large karst fields (Sinjsko, Petrovo, Kosovo and Kninsko). During our previous toxicological study near gypsum mine in Kosovo field (Mihaljević et al., 2011), it was observed that macrozoobenthos samples included a multitude of mechanically damaged *Gammarus balcanicus* specimens, displaying a very soft exoskeleton. These individuals were exclusively distributed in all of the investigated stations downstream of the gypsum mine where higher environmental strontium concentrations were also determined in the environment (both in water and sediment).

Species of the genus *Gammarus* have been used in numerous ecotoxicological studies because they are net accumulators of trace metals (Rainbow and Moore, 1986; Rainbow, 2002) and are considered good biomonitors of environmental metal availabilities in general (Fialkowski et al., 2003). Gammarids are integral to freshwater food webs as they are the main link between detritus and fish and the reduction of their abundance can have a detrimental effect on the stream community structure (MacNeil et al., 1997; Kunz et al., 2010).

The biological effects of strontium are related to its chemical similarity to calcium. Calcium balance is essential for crustaceans:

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