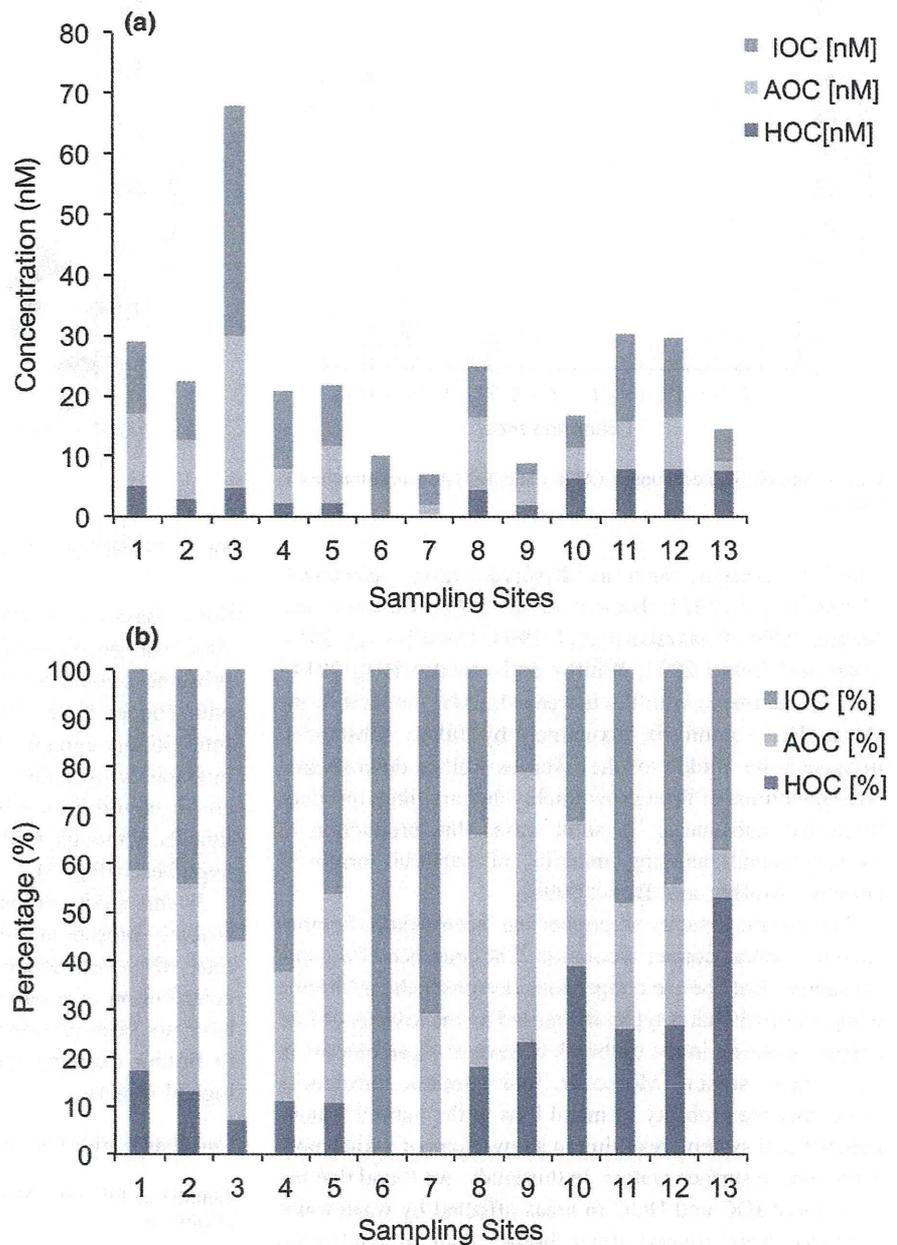
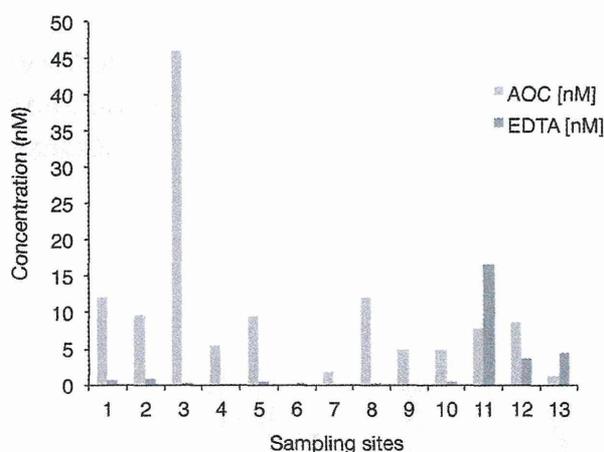


**Fig. 5** Hydrophobic organic copper (HOC), anionic ligated copper (AOC), and inorganic copper (IOC) concentrations (a) and percentage of each substance (b) in river water



dissolved copper ions (Buck et al. 2007). These organic materials were also shown to have high affinities with copper. Although organic substances dissolved in aquatic environments are important factors in the determination of Cu speciation, the presence of anionic complexes has not been previously considered. The presence of AOC in river water was first identified in this study. Because the origin of the AOC was presumed to be chelated copper ions, the concentration of EDTA was compared with that of AOC (Breault et al. 1996). In several regions in Japan, EDTA is known to be a limiting factor in the growth of algae (Nanjo et al. 2000). We found that three sites in the Sakai River

contained amounts of EDTA that were comparable to those of AOC (Fig. 6). It is possible that these water samples contain EDTA–Cu complexes as the AOC species (Rozañ and Benoit 1999). In some cases, the EDTA concentrations were much higher than those of AOC, probably due to metals that can easily bind to EDTA, such as Fe in drainage water, or the presence of some organic ligands that can form non-ionic copper complexes. The influence of the latter process, however, will be a minor one. However, at the other sites, the EDTA concentrations were quite low, and therefore, the presence of AOC cannot be accounted for by EDTA alone. Other possible chelating compounds



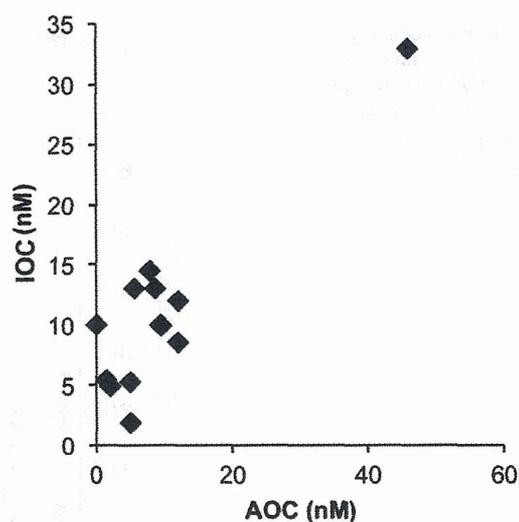
**Fig. 6** Anionic ligated copper (AOC) and EDTA concentrations in river water

could be present, such as dissolved fulvic substances (Ahmed et al. 2013; Breault et al. 1996; Cabaniss and Shuman 1988; Chakrabarti et al. 1994; Cottrell et al. 2014; Sodré and Grassi 2007; Whitby and van den Berg 2014). The IOC decrease with the increase in AOC content in the Ogino River could be explained by fulvic substances, because in the middle of the river, as well as downstream, large quantities of flora grow thickly and are likely produce fulvic-like substances. In some cases, the production of strongly metal-chelating material of bacterial origin is possible (Moffett and Brand 1996).

The aquatic toxicity of copper has been clearly demonstrated to reflect copper speciation. The presence of organic substances that chelate copper ions decreases the inhibition of algal growth that is typically caused by the toxicity of free copper, resulting in the outbreak of several algae blooms in the summer season. Moreover, these organic substances could alter the mobility of metal ions in the natural water-geochemical system, resulting in an overload of toxic heavy metal ions in surface waters. In this study, we found that the amounts of IOC and HOC in areas affected by wastewater discharge were several times higher than in unaffected waters. In such areas, EDTA-Cu<sup>2+</sup> can be present as AOC. These results illustrate that the burden of copper on the environmental system from drainage water may not be negligible with regard to aquatic biota toxicity, even though the concentration of IOC downstream from the sewage plants was lower than the typical toxic dose.

## Conclusions

Anionic chelated copper was initially reported in this study, and EDTA was suggested as a possible candidate ligand, in part, especially in locations affected by drainage water



**Fig. 7** Correlation of AOC and IOC in river water tested in this study

flows. Based on the single sample examined in this study, AOC was not found in clear upstream river water. Fulvic substances could be candidates for the production of AOCs with copper in the middle and lower sites of the rivers. Interestingly enough, the AOC and IOC concentrations are moderately correlated, possibly because these complexes are in equilibrium with the free copper ions and organic ligands, although further studies are needed to clarify this hypothesis (Fig. 7).

In this study, anionic ligated copper species were consistently present at 2–46 nM, amounting to 9–56 % of the total dissolved copper in the samples, with an average contribution of around 30 %. Because these AOC species have not been reported in the published literature, we plan to further examine their geochemical behaviors and biological effects.

## Compliance with Ethical Standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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