

MALACHITE GREEN IN SUSPENDED PARTICULATE MATTER AND SURFACE SEDIMENTS IN GERMANY

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Abstract

Besides its application as dyestuff malachite green is applied as veterinary drug in ornamental pisciculture. Due to its potential carcinogenicity it is forbidden to be used in the EU for commercial food fish farming. Nevertheless, contaminations of aquatic species have been reported. Therefore, suspended particulate matter (SPM) samples from the Environmental Specimen Bank Germany (ESB) from 2007 and 2010 and surface water sediments from the Berlin area representing similar time ranges have been analyzed for malachite green (MG) and leuco malachite green (LMG). Solely malachite green but no leuco derivative has been detected with increasing concentrations of up to 543 ng/g dry weight in the 2010 SPM samples. Sediment samples partly influenced by sewage treatment plant effluents revealed moderate concentrations. This study indicated the relevance of malachite green as sedimentary pollutant and points to an environmental stability also under natural anaerobic conditions.

Zusammenfassung

Malachitgrün wird neben der Verwendung als Farbstoff als Tierarzneimittel in der Zierfischzucht eingesetzt. Aufgrund seiner potenziellen Karzinogenität ist eine Anwendung in der kommerziellen Speisefischzucht in der EU verboten, dennoch sind Kontaminationen von Speisefisch publiziert. Aus diesem Grund wurden Schwebstoffe der Umweltprobenbank des Bundes (UPB-UBA) aus den Jahren 2007 und 2010 sowie Oberflächensedimente aus dem Berliner Raum, die dem Zeitraum 2010-11 entsprechen, hinsichtlich Malachitgrün und Leukomalachitgrün, dem reduzierten Metaboliten, untersucht. Es wurde nur Malachitgrün mit bis zu 543 ng/g Trockensubstanz bei insgesamt gestiegenen Gehalten in den Proben aus dem Jahre 2010 nachgewiesen. Die Proben aus dem Berliner Raum, die durch Klärwerkseinleitungen geprägt sind, wiesen moderate Kontaminationen auf. Die Arbeiten belegen eine Kontamination von Schwebstoffen und Oberflächensedimenten und eine Persistenz von Malachitgrün unter anoxischen Bedingungen.

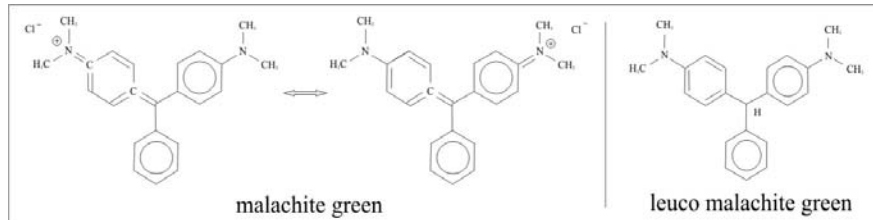
Keywords: Malachite green, ESB, SPM, surface sediment

1) Introduction

Malachite green is intensively used as dyestuff worldwide. Due to its antifungal, antiparasitic and antibacterial properties malachite green is also applied as veterinary drug in ornamental pisciculture, but is for reasons of potential carcinogenicity not allowed by EU law to be applied in commercial food fish farming (Council Regulation **2377/90/EC**). However, due to the low costs it is utilized in China and Taiwan in aquaculture. The application is based on the salt formed by substituted triaryl methane cation and oxalate or chloride as corresponding anion. Malachite green has a high water solubility of 40 g/L (25°C) and, consequently, a low

particle reactive sorption ($\log K_{ow} = 0.62$). Hence, malachite green tends to remain in the water after release to the environment [Arnold et al. 2009].

Under anaerobic environmental conditions malachite green is known to be transformed in a reversible reaction into the corresponding leuco compound.



Leuco malachite green exhibits a much lower water solubility and a higher tendency to adsorb to suspended particulate matter and sediments ($\log K_{ow} = 5.72$). Interestingly, leuco malachite green is also the first metabolite formed in fishes. Here, the lower hydrophilicity leads to an enhanced accumulation in fat and muscle tissues. As a consequence, malachite green but to a higher extent leuco malachite green has been detected in fishes, in particular from China (Schuetze et al. 2008), Taiwan and further countries with extended fish farming. Analysis of fish samples from the German Environmental Specimen Bank (ESB) [Apel et al. 2010] and eel samples from the Berlin area in 2007 [Schütze et al. 2008] revealed partially elevated concentrations of malachite green and its leuco derivative, e.g. up to $0.765 \mu\text{g/kg}$ d.w. in samples from the Teltow Canal (Berlin). Most of the elevated concentrations were measured near effluents of sewage treatment plants. For comparing the level of contamination with particulate samples from the same regions in 2011 sediment cores from the Berlin area and SPM samples from the ESB (dated 2007 and 2010) were analyzed for MG and the transformation product LMG.

2) Materials and methods

In this study two different sample sets have been investigated. A first set consisted of 21 samples of suspended particulate matter (SPM) obtained from the Environmental Specimen Bank Germany. Generally, SPM samples for the ESB are taken on a monthly basis by means of sedimentation boxes [Schuberth et al. 2012], from which yearly homogenates are created. The samples investigated in this study derived from 2007 and partially 2010 (not all 2010 samples were available, s. tab. 1). Sampling locations of the ESB are carefully selected to represent different ecosystems and not to be influenced by local sources.

A second set of sediment samples were obtained from Spree and Havel river sections located in the urban area of Berlin. These samples were collected according to Schulze et al. [2007] with liner coring for sediments. Six cores were drilled with a length of 30 cm, which were subdivided into 2 cm layers. For this study the top layers (0-2 cm, up to 2 years old material) and the layer 4-6 cm representing a sediment age of approx. 5 to 6 years were analyzed.

For the determination of malachite green and leuco malachite green the freeze-dried particulate matter was extracted with acetonitrile and hydroxylamine according to Weiß et al. [2010]. Quantitative analyses were performed on a LC-MS/MS system operating in ESI⁺ mode. Chromatographic separation was carried out on a Luna 3 μm PFP column (150 x 3.0 mm) with isocratic elution using MeOH/H₂O (90/10) with a flow of 0.2 mL/min. Quantification

was performed with an internal standard calibration using deuterated (D5) standards. The limit of quantification (LOQ) was approx. 3 ng/g d.w..

Tab. 1: Amount of malachite green and leuco malachite green detected in suspended particulate matter from various German rivers as well as in sediment cores of Spree and Havel rivers in the urban area of Berlin

Sample	Layer / Year	TOC [%]	MG [ng/g d.w.]	LMG [ng/g d.w.]
Sediment cores Berlin area				
Lake Kleiner Wannsee	0 - 2 cm	n.m.	< LOQ	< LOQ
	4 - 6 cm	11.8	< LOQ	< LOQ
Lake Griebnitz	0 - 2 cm	8.2	25.6	< LOQ
	4 - 6 cm	n.m.	< LOQ	< LOQ
Teltow Canal - STP Ruhleben	0 - 2 cm	1.0	4.7	< LOQ
	4 - 6 cm	n.m.	< LOQ	< LOQ
Teltow Canal - STP Wassmannsdorf –	0 - 2 cm	0.3	< LOQ	< LOQ
	4 - 6 cm	n.m.	< LOQ	< LOQ
Inflow Erpe into Spree river	0 - 2 cm	19.6	< LOQ	< LOQ
	4 - 6 cm	n.m.	< LOQ	< LOQ
Lake Müggel	0 - 2 cm	11.5	< LOQ	< LOQ
	4 - 6 cm	n.m.	< LOQ	< LOQ
Suspended particulate matter				
Elbe river E1	2007	n.m.	< LOQ	< LOQ
Elbe river E2	2007	5.4	12.7	< LOQ
	2010	5.5	88.8	< LOQ
Elbe river E3	2007	3.9	19.8	< LOQ
	2010	4.5	186	< LOQ
Elbe river E4	2007	4.5	38.5	< LOQ
	2010	5.5	128.4	< LOQ
Elbe river E5	2007	n.m.	< LOQ	< LOQ
	2010	3.9	166	< LOQ
Saale river Sa1	2007	n.m.	< LOQ	< LOQ
	2010	6.5	178	< LOQ
Mulde river M1	2007	n.m.	< LOQ	< LOQ
Saar river S1	2007	n.m.	< LOQ	< LOQ
Saar river S2	2007	n.m.	< LOQ	< LOQ
Rhein river R1	2007	3.6	24.8	< LOQ
	2010	4.6	168	< LOQ
Rhein river R2	2007	n.m.	< LOQ	< LOQ
	2010	4.3	543	< LOQ
Rhein river R3	2007	n.m.	< LOQ	< LOQ
	2010	3.9	267	< LOQ
Rhein river R4	2007	n.m.	< LOQ	< LOQ

n.m. = not measured

3) Results and discussion

Table 1 summarizes the results for malachite green in suspended particulate matter attributed to the sampling locations of the ESB for both 2007 and 2010 samples. Concentrations ranged between LOD (1 ng/g d.w.) and 543 ng/g. For the samples from the ESB remarkable increases between 2007 and 2010 are depicted, even at sampling locations with no detection in 2007. On the contrary, only in a few core samples from the Berlin region malachite green was detected. The detection was restricted to the top layers of only some samples and represents only a low level of contamination. In Lake Griebnitz the maximum concentration in the surface sediment was 26 ng/g d.w.. Polluted sampling locations in Berlin correspond well with the areas in which fish samples also revealed a malachite green contamination (Schütze et al. 2008). These areas are highly influenced by municipal sewage effluents. To the best of our knowledge, these are the first data on malachite green including its leuco form in particulate matter of natural surface water systems. Additionally, our results allowed some further insight into the environmental fate of malachite green in the aquatic environment. Firstly, there was no correlation ($R^2 = 0.01$) with TOC values indicating an expected low tendency for enhanced adsorption to lipophilic matter. Secondly, no leuco malachite green was detected in any sample despite anoxic conditions were evident already in the first mm-layers of the sediment cores. This supports the results by Weiß et al. 2010, who reported a formation of leuco malachite green starting after 4 weeks in laboratory experiments but with low concentrations at significantly elevated concentrations of MG. Hence, our results indicate an environmental stability of malachite green also under natural anaerobic conditions. Thirdly, the origin of the detected recent emissions of malachite green cannot be attributed to primary emission sources so far. The dominant occurrence in municipal waste waters and aquatic systems directly influenced by those effluents indicate a more common application. Noteworthy, some reports on illegal use of malachite green as veterinary drug in the EU are available [Halme et al. 2007, Little et al. 2012, Nebot et al. 2013].

4) Conclusions

Analysis of suspended particulate matter from different German rivers as well as sediment cores from rivers of the Berlin region provided first data on malachite green in riverine particulate matter. Interestingly, a significant increase of malachite green contamination between 2007 and 2010 by a factor of up to 10 has been revealed. The more lipophilic leuco compound has not been detected in any sample, hence this derivative has no environmental relevance in sedimentary aquatic systems.

Generally, this study indicated the relevance of malachite green as sedimentary pollutant and points to an environmental stability also under natural anaerobic conditions. However, due to its limited lipophilicity it remains unclear whether sediments act as sink or source of malachite green contamination.

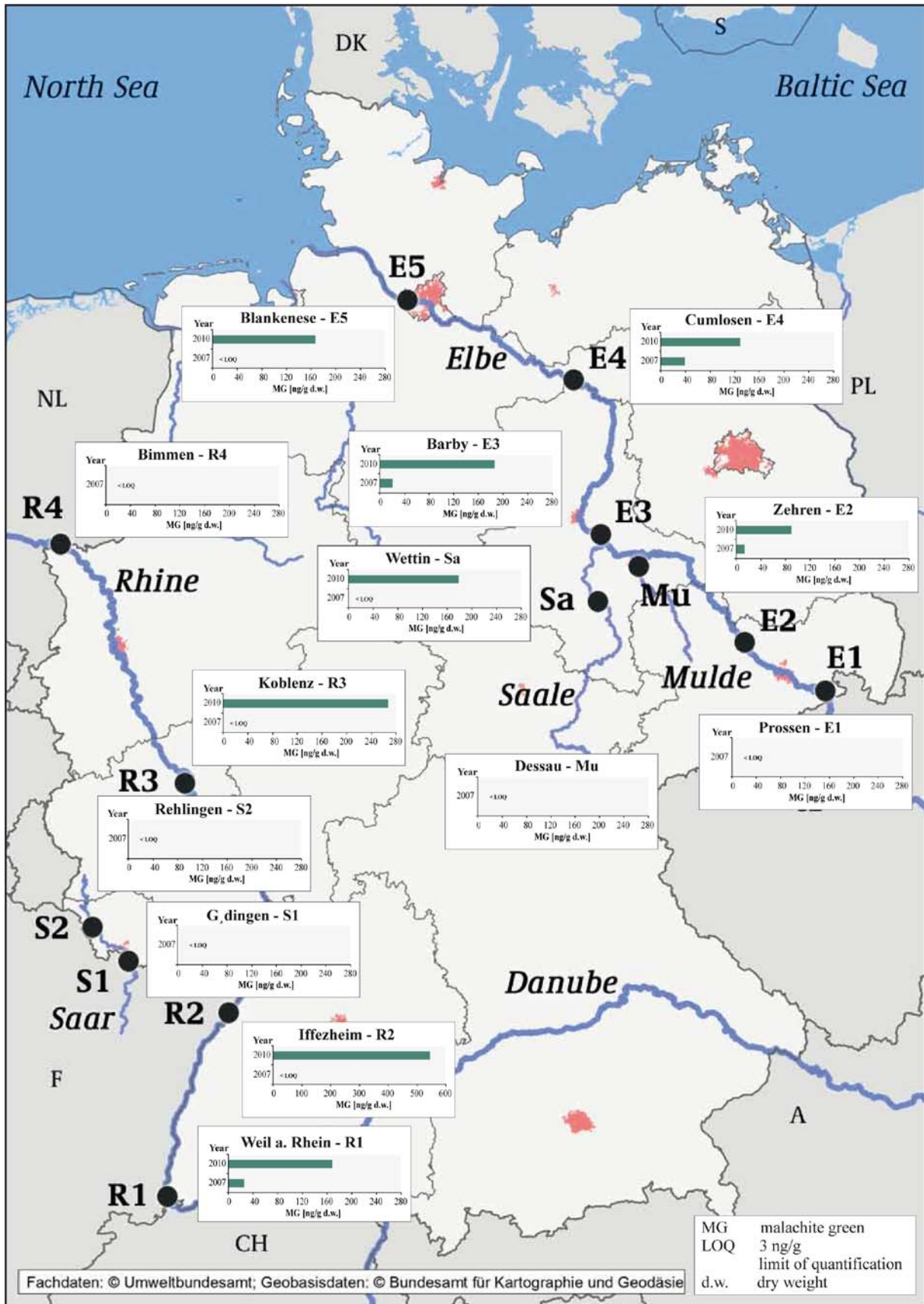


Figure 1: Map of the sampling locations of the ESB and results of the MG-analysis.

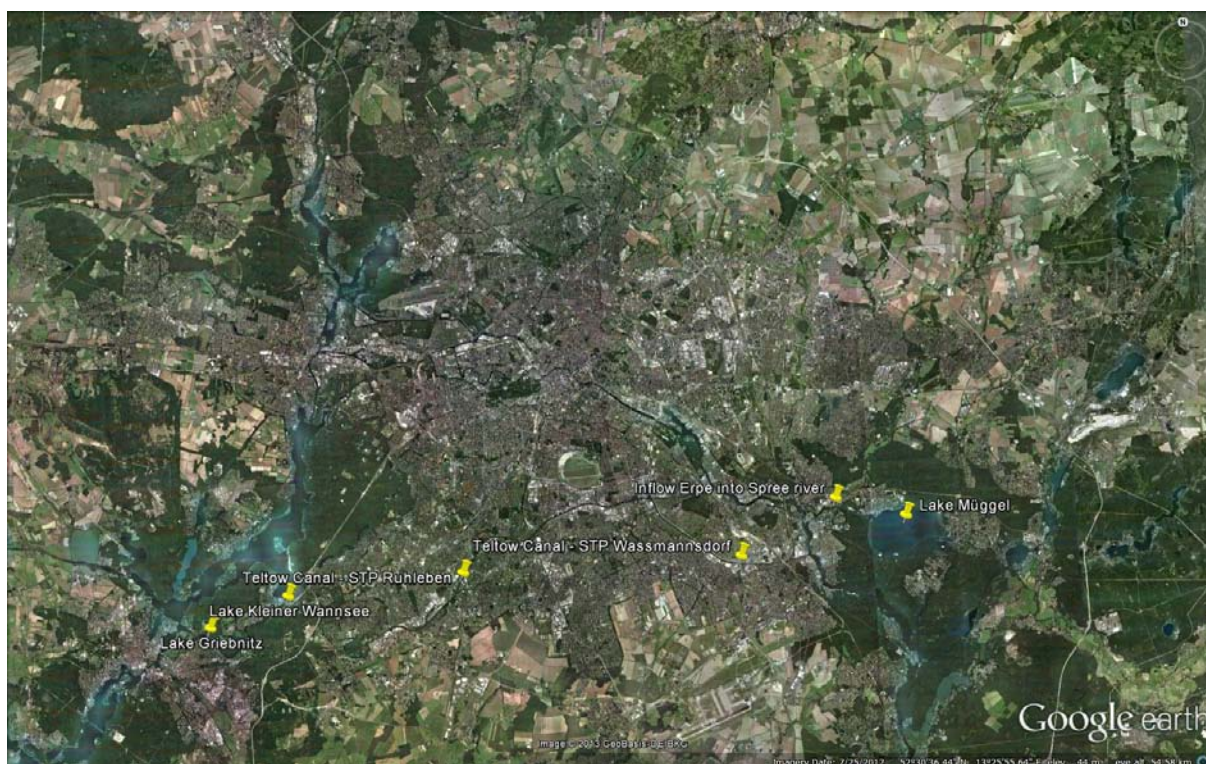


Figure 2: Map of the sampling locations in the Berlin area.

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