



Mutagenic effects of madder root in textile dyeing processes

Christoph Hafner, Ismene Jäger, Hydrotox GmbH, D-79111 Freiburg
Klaus Schneider, FoBiG GmbH, D-79098 Freiburg
H. Iznaguen, Universitätsklinikum Hamburg Eppendorf, D-22527 Hamburg

Introduction

Madder roots (*Rubia tinctorum*) have been used for textile dyeing for many centuries up to the present. It is known that extracts of madder roots contain anthraquinones such as lucidine, whose mutagenic and carcinogenic effects have been described in various publications. To determine the possible environmental and toxicological risks in the dyeing process and for dye house workers and consumers wearing textiles dyed with madder root, bacterial reverse mutation assays with *Salmonella typhimurium* (Ames Test) were performed. Dyeing processes with roots of different origin (Bhutan and Iran) were examined. Root extracts, dyeing baths, wastewater samples and the dyed wools were tested.

Methods

Bacterial Reverse Mutation Assay (Ames Test)

Tests were performed according to the OECD guideline 471 and the Directive 2000/32/EG, B. 13/14 with selected strains (TA98, TA100 and TA1537) with and without metabolic activation (S9). A doubling of the number of revertants compared to the negative control (spontaneous mutation rate), which corresponds to an induction rate (IR)>2, was interpreted as a mutagenic effect of the sample. In testing a concentration-series any dose-dependent effect should be detectable. IR<1 indicates a bacteriotoxic effect of the sample; an interpretation as regards mutagenic effects thereby is not possible.

Sample preparation

A) Organic root extracts

B) Organic extracts of pressed cake

C) Organic extracts of the wool

1 - 8 g DS (A and D), 5 g DS (F) of the solid samples were extracted by Soxhlett with ethyl acetate and ethyl alcohol. Samples were concentrated to dryness in a rotary evaporator and then taken up in 1 ml DMSO for the Ames Test; 20 and 40 µl DMSO extract were tested; (DS = dry substance)

D) Aqueous dyeing bath

Adjusting pH-value to 7.0 +/- 0.2 with subsequent sterile filtration (0.45 µm); 1,000 µl was tested;

E) Organic extracts of the four aqueous root extracts

F) Organic extracts of the aqueous dyeing baths

G) Organic extracts of the residual liquids (sewage water)

16 - 20 ml each was extracted with ethyl acetate; Samples were concentrated to dryness in a rotary evaporator and then taken up in 1 ml DMSO for the Ames Test; 20 and 40 µl DMSO extract were tested;

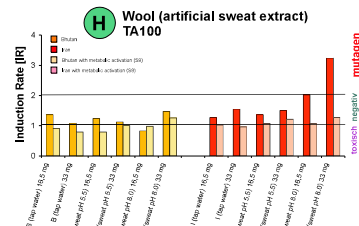
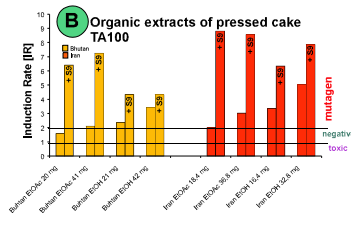
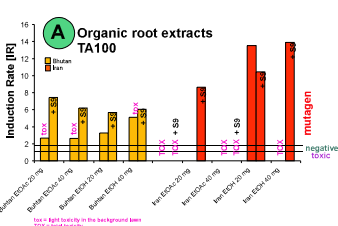
H) Eluates of the dyed wool with artificial sweat

1g wool was eluated for 24h with acidic artificial sweat solution (according to DIN 54 020 with L-arginine-mono-hydrochloride instead of histidine); Adjusting pH-value to 7.0 +/- 0.2 with subsequent sterile filtration (0.45 µm); 1,000 and 500 µl were tested;

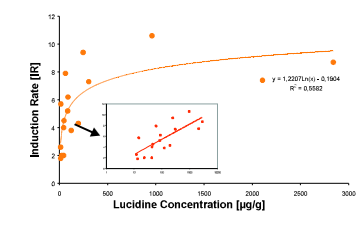
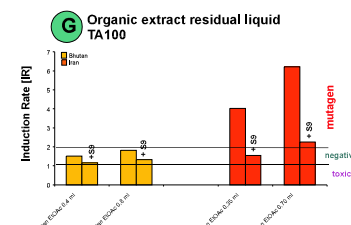
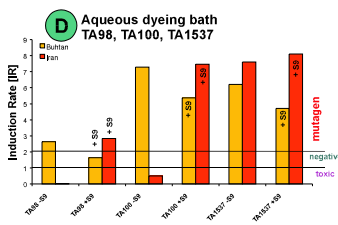
Lucidine analysis in the organic extracts

Analytics were performed with HPLC according to Westendorf et al. 1988;

Results



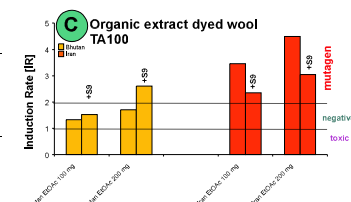
Sample	amount of sample in 1 ml DMSO	Lucidine Concentration (µg/g light DMSO)	Lucidine Concentration (µg/g - µg/ml) active sample
1 st aqueous extract Bhutan	16 ml	1354	84.6
1 st aqueous extract Iran	17 ml	1052	61.9
2 nd aqueous extract Bhutan	17 ml	3331	195.9
2 nd aqueous extract Iran	17 ml	16319	959.9
3 rd aqueous extract Bhutan	15 ml	649	43.3
3 rd aqueous extract Iran	16 ml	3530	245.6
4 th aqueous extract Bhutan	17 ml	296	23.3
4 th aqueous extract Iran	17 ml	747	43.9
dyeing bath Bhutan	16 ml	1950	121.9
dyeing bath Iran	17 ml	251	14.8
pressed cake Bhutan	1.02 ml	310	303.9
pressed cake Iran	0.92 ml	n.d.	n.d.
residual liquid Bhutan	20 ml	270	13.5
residual liquid Iran	17.5 ml	1540	88.0
madder root origin Bhutan	1.0 g	2108	2108.0
madder root origin Iran	1.0 g	2840	2840.0
dyed wool Bhutan	5.0 g	61	12.2
dyed wool Iran	5.0 g	236	47.2



Conclusions

- In most samples mutagenic effects could be detected in the Ames Test
- Root extracts induced the highest number of revertants, extracts of dyed wool the lowest
- The effects correlated with the lucidine content detected
- Mutagenicity of madder root origin Bhutan was lower than of origin Iran
- Artificial sweat extracts of dyed wool with madder root origin Iran indicate mutagenic activity
- A potential risk for dye house workers and consumers can be stated
- An environmental risk by wastewater can not be excluded

	Bhutan		Iran	
	TA98	TA100	TA98	TA100
First aqueous boiling extract	1.36	0.89	5.23	2.95
Second aqueous boiling extract	1.29	1.12	4.36	2.64
Third aqueous boiling extract	0.84	1.20	1.98	1.24
Fourth aqueous boiling extract	0.79	0.97	1.95	1.56
Dyeing bath consisting of the four boiling extracts	2.67	1.75	3.81	1.95



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The project was finished in June 2003. Further information is presented at www.hydrotox.de (downloads).



Rubia tinctorum (Madder)

