The Mutagenic Potential of Madder Root in Dyeing Processes of the Textile Industry

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Abstract

The roots of *Rubia tinctorum* L. have a long tradition in dyeing processes of textiles from centuries ago until present. The colouring principles belong to hydroxyanthraquinones. Concern arose because several of these compounds were recognised as mutagenic in vitro and even carcinogenic in rodents. To assess the possible risk for humans caused by textiles, the mutagenicity was investigated using two madder root samples of different origin (Iran and Bhutan) along the entire dyeing process from root extracts to the dyed wool using the *Salmonella*/microsome test with the strains TA 98, TA 100 and TA 1537 (Ames Test). Significant mutagenic effects could be detected in madder root extracts and also in the final product, the dyed wool. Madder root from Iran showed considerably higher mutagenic responses compared to samples from Bhutan. Analytical investigations of the extracts by HPLC showed the presence of a spectrum of anthraquinones typical for madder root. Three mutagenic compounds, lucidine, rubiadine and purpuroxanthine, together with non-mutagenic alizarine could be detected. The mutagenic response of the different samples was positively correlated with the concentration of the mutagenic anthraquinones and lucidine in particular. Based on these investigations a risk for dye house workers and consumers of textiles dyed with *Rubia tinctorum* must be anticipated.

1 1. Introduction

Madder, the root of *Rubia tinctorum* L., has a long tradition as a dyestuff because of its bright red colour. The red pants of Napoleons army and the red coats of the English soldiers in the

18th/19th century were all dyed with madder. After the synthetic availability of alizarine in 1868 madder lost most of its importance as dye stuff for textiles, although its use never ceased totally since today. In recent years madder root retrieves importance in the context with clothes exclusively dyed with natural dyes. Madder roots were also used in traditional medicine as a drug against kidney and bladder stones. All pharmaceutical products based on madder root were withdrawn from the European market in 1992 (BAnz., 1992) after several investigations became available to demonstrate the genotoxic potential of anthraquinones present in these products (e.g., Yasui and Takeda, 1983, Westendorf et al. 1988, Marec et al., 2001, Tikkanen et al., 1983a, Tikkanen et al. 1983b, Westendorf et al. 1990, Mori et al. 1990, Kawasaki et al. 1992, Westendorf et al., 1998, Wölfle et al.,1990, Blömeke et al. 1992, Poginsky et al. 1991, Marczylo et al. 2000). An overview gives Derksen (2001).

Although madder roots contain several hydroxyanthraquinones which are mutagenic in bacterial and mammalian systems, lucidine (1,3-dihydroxy-2-hydroxymethyl,9,10-anthraquinone) was demonstrated to be most potent. It was shown that lucidine is able to form DNA adducts *in vitro* and *in vivo* after treatment of tissue culture or laboratory animals with the compound (Poginski et al. 1991). A correlation could be demonstrated between the formation of lucidine-DNA adducts and the formation of liver and kidney tumours in rats treated with madder roots in their diet for a period of two years (Westendorf et al., 1998). Recently the EU initiated a research project to investigate the possible risk of dyes used in the textile industry (Identification and Substitution of Mutagenic Dyes in Textile Finishing, QLK4-CT-200-70158) (Jäger et al. 2005, Schneider et al. 2004, Jäger et al. 2004). Among the five plant dyes investigated in this study, only madder root was known from the literature to contain genotoxic ingredients. In the present study the mutagenic potential of madder root solutions used in the dyeing process of textiles as well as the final product, the dyed wool was investigated in the *Salmonella*/microsome assay (Ames test) and the results were compared to the composition of hydroxyanthraquinones analyzed by HPLC analysis.

2 2. Materials and Methods

2.1 Test compounds

An overview of the samples investigated is given in Fig. 1. Samples were taken directly from the standard dyeing process in a textile finishing company. Samples consist of unprocessed madder roots, four sequentially prepared root extracts with boiling water, the combined extracts used for dyeing of textiles, pressed cakes of madder roots after water extraction, the residual dyeing bathes after use and the final products the madder dyed wool. All samples were stored in 250 ml screw-caps PE-flasks in the freezer at -21°C until further use. Wool strands of 20g each, wrapped in aluminium foil, were also stored in the freezer.

2.2 Reagents

Alizarine was purchased from Carl Roth GmbH, Karlsruhe, Germany. Lucidine and Xanthopurine were synthesized according to Murti et al. (1970) modified by Poginsky (1989). Rubiadine was synthesized according to Blömeke (1991).

For the cultivation of *S. typhimurium* strains in the Ames Test Nutrient Broth No. 2 (Oxoid, Basingstoke, England) was used. Agar plates and soft agar were prepared with Bacto-Agar (Becton, Dickinson & Company, Sparks, U.S.A.) and Rat liver S9 Aroclor 1254 induced was purchased by Moltox Inc. Boone, U.S.A.. The positive control substances 2-nitrofurantoine, 4-nitro-1,2-phenylendiamine and 2-aminoanthracene were purchased by Sigma Chemical, St. Louis, U.S.A..

2.3 Sample preparation

The investigation of the aqueous samples in the bacterial mutagenicity test was performed directly after adjusting the pH value to 7.0 ± 0.2 and subsequent sterile filtration through a syringe filter (0.2 µ). Organic extracts of the aqueous phases were prepared by subsequent liquid extraction of 16-20 ml with equal amounts of ethyl acetate. The extraction was repeated 3-7 times until the organic phase was colourless. The combined organic phases were evaporated in a rotary evaporator to dryness and dissolved in 1 ml DMSO. The solid samples (unprocessed roots, root press cakes, wool) were soxhlett extracted with ethyl acetate and subsequently with ethyl alcohol. After evaporation of the solvent the residues were dissolved in 1 ml DMSO per g dry weight. The wool was also extracted with artificial sweat in a modification of ISO 105-E04 (1994). Instead of histidine mentioned in this guideline, arginine had to be used because histidine would have influenced the bacterial mutagenicity test. Two artificial sweat solutions (pH 8.0 and pH 5.5) were prepared by dissolving 0.5 g/l L-arginine-monohydrochloride. Tap water was used as control. The wool (1g) was shaken in 30 ml of these solutions in a shaking incubator at 130 rpm for 24 hours at room temperature. Extracts were filtered; the pH-value was adjusted to 7.0 ± 0.2 and sterilized using a syringe filter (0.2 µ).

2.4 Bacterial mutagenicity test

The *Salmonella*/microsome assay was performed in accordance to the OECD guideline 471 of July 1998 and the Council Regulation No. 2000/32/EG, B. 13/14 of May 2000. Strains TA98, TA 100 and TA 1537 were included in the survey. The organic extracts of madder roots, residues of roots after extraction and residual liquid as well as wool extracts were investigated exclusively in TA100 because in the investigations of the dyeing baths and boiling extracts this strain responded more strongly and specifically showed the mutagenic activity of lucidine. Revertant colonies were counted with a semiautomatic colony counter. Samples were evaluated positive if the number of revertants was doubled in comparison to controls and/or a positive dose relationship occured.

2.5 Chemical analysis of anthraquinones

The organic extracts investigated in the bacterial mutagenicity test were analysed for the presence of anthraquinones by HPLC-analysis. A Thermo-Finnigan HPLC-system equipped with a gradient pump, an auto sampler and a diode-array-detector was used. 20 µl of the DMSO solutions were injected on a RP-18-column and eluted with a flow rate of 2 ml/min. and a gradient of acetonitril (A) and 0.1% acetic acid (B): (0-5 min: 20% A, 5-15 min: 20-45% A, 15-20 min.: 45% A, 20-30 Min.: 45-100 % A, 30-40 min.: 100 % A). Quantification of the anthraquinones in the extract was performed with the external standard method by use of identical reference compounds.

3 Results

3.1 Mutagenicity

3.1.1 Aqueous dyeing bath and aqueous boiling extracts

With the original aqueous dyeing baths (DBB, DBI) mutagenic responses could be observed with all three *Salmonella typhimurium* tester strains (TA 1537, TA 100 and TA 98) (Tab. 1). The madder sample from Iran showed the highest responses in the absence of S9 mix, whereas the opposite effect was observed with madder sample from Bhutan. The highest induction rate of 8.2 was obtained with the dyeing bath prepared from madder samples of Bhutan in strain TA1537 in the presence of S9 mix. Without S9 activation the Bhutan dyeing bath was very toxic.

Ethyl acetate extracts of the dyeing bath (DBB, DBI) showed mutagenic effects in TA100 for both origins, whereas only marginal increases of the mutation rate were observed with strain TA98 (Tab. 2). Similar results were observed for the aqueous boiling extracts 1-4 (AE1 – AE4, Tab. 2). Highest responses occurred with strain TA100 in the absence of S9 mix. In all four fractions the samples from Iran showed higher responses compared to samples from Bhutan. The peak of mutagenicity was obtained in the second boiling extracts of both origins, showing a slow mobilisation of the mutagenic anthraquinones from the root material. Extracts of the residual liquid of the dyeing bath from the Iran sample after use (RLI, Tab. 3) still showed mutagenic responses in TA100 with and without S9 mix (IR=6.2), whereas residual liquid from Bhutan was not mutagenic in TA100.

3.1.2 Mutagenicity of madder roots before and after preparation of the dyeing bathes

Strong mutagenic effects were observed for all extracts of madder root (MR, Tab. 3) with induction rates between 5.7 – 13.9 (TA100 with S9 mix). Samples from Iran, again, were more effective compared to samples from Bhutan. In addition toxic effects occurred especially in the tests without metabolic activation and in the extracts with ethyl alcohol. Samples from Iran were more toxic than samples from Bhutan.

The root residues (pressed cake) after preparation of the boiling water extracts (RR, Tab. 3) were also extracted with ethyl acetate and ethyl alcohol. The mutagenicity of these samples was only moderately reduced compared to the extracts prepared from the original roots. The induction rates ranged between 4.3 and 8.8 (TA100 with S9 mix). Again samples from Iran resulted in higher induction rates. Toxicity observed with rooot extracts in TA100 without metabolic activation, masking mutagenic effects, dissappeared in the extracts of root residues.

3.1.3 Mutagenicity of dyed wool

Dyed wool was extracted with ethyl acetate, tap water, artificial acidic sweat (pH 5.0) and alkaline sweat (pH 8.0). As demonstrated in Tab. 4 ethyl acetate extracts of the wool dyed with madder root from Iran (DWI EtOAc) was mutagenic in strain TA100 with and without S9 mix. Wool extracts dyed with madder from Bhutan (DWB EtOAc) showed mutagenic effects only with metabolic activation. Wool extracts prepared neither with tap water (DW tw) nor with acidic sweat (DW AcS) showed a mutagenic response in the concentrations tested. Positive results were obtained with alkaline artificial sweat (DWI AlS) extracts from wool of origin Iran.

3.2 Chemical analysis

The anthraquinones (AQ) composition of the different samples prepared from madder roots was analysed. Fig. 2 shows chromatograms from extracts of unprocessed madder roots, from dyeing baths and from dyed wool of origin Bhutan and Iran. The ethyl acetate extracts of the roots show the typical pattern of AQ as described earlier by Westendorf et al. (1998). A glycosidic fraction containing the primverosides of alizarine and lucidine eluted with retention times of 8.9 and 9.3 minutes. Among the free aglycones lucidine (RT= 16.2), alizarine (RT = 17.8), purpuroxanthine (RT = 21.8) and rubiadine (RT = 248) were clearly identifiable. Tab. 5 presents the AQ concentrations calculated from the peak areas of the chromatograms shown in Fig. 2. Comparing the root extracts from Bhutan and Iran almost equal amounts of lucidine, and purpuroxanthine occurred, whereas alizarine concentration was almost five-fold higher in madder roots from Iran. Only small amounts of purpuroxanthine occurred in the root extracts of both origins. The extracts from the dyeing baths and the dyed wool reflected the AQ composition of the root extracts. In the wool dyed with root extracts from origin Iran the alizarine concentration is almost 26-fold higher than in samples dyed with roots from origin Bhutan. On the other hand with the dyeing baths AQ concentrations were higher in the samples from Bhutan origin. The concentration of rubiadine was higher in all samples from madder root of origin Bhutan than in the samples from Iran.

Lucidine, because of its toxicological profile, is the most critical AQ present in madder roots and the only AQ being mutagenic in *Salmonella* strain TA100. Therefore, the number of revertants obtained in the *Salmonella* mutagenicity assay with strain TA100 was correlated with the lucidine concentration for all extracts representing the whole dyeing process. Fig. 3 illustrates the significant positive correlation between the lucidine concentration of the pooled

samples and the revertants induced in the strain TA100. A maximum induction rate of 8-10 was achieved with a lucidine concentration of 700 µg/ml approximately. Higher concentrations were toxic.

4 Discussion

Synthetic and natural AQ are an important group of dyes used to dye textiles and foodstuff. Although many AQ are mutagenic in the *Salmonella*/microsome assay, very few are genotoxic in mammalian systems (Brown & Dietrich 1976; Westendorf et al. 1990, Kawasaki 1992, Tanaka 2000). Among the AQ present in madder root, a traditional natural dyestuff, lucidine, rubiadine and purpuroxanthine are mutagenic in bacteria and mammalian cells. It could be demonstrated that lucidine is able to form DNA adducts *in vitro* and *in vivo* (Poginsky et al. 1989). ACI rats receiving a diet containing 1% or 10% madder root showed an increase of the incidence of benign and malignant tumours in liver and kidneys (Westendorf et al., 1998). The tumour incidence was related to the concentration of lucidine-DNA adducts in these organs. Because of the genotoxic potential of several AQ present in madder root and lucidine in particular, its contact with humans is of concern. It was, therefore of interest, to follow these compounds during the dyeing process from the preparation of the dyeing bathes to the dyed wool. Analysis of two different madder samples used in the dyeing industry (origin Iran and Bhutan) showed significant differences in the composition of AQ. The sample of Iran contained mostly alizarine. This AQ is most important for the dyeing process and is of low toxicity. Lucidine is the most critical AQ present in madder roots and the only AQ being mutagenic in *Salmonella* strain TA100. The lucidine concentration in the different samples was therefore given special consideration.

Significant mutagenic effects could be detected in the different madder root extracts and even in the final product, the dyed wool. In all samples lucidine could be detected. Especially the root extracts contained lucidine concentrations in the mg/ml range followed by the aqueous boiling root extracts No. 2 (AE2). An increase of the mutation rate with increasing lucidine concentrations was observable. Also the alizarine concentration in the dyeing bath which is the main dye component (Derksen, et al. 2003), correlates positively with the lucidine concentration, which is responsible for the unwanted mutagenic effect. Above all, the demonstration of the mutagenic potential in the extracts from dyed wool indicates that in the dyeing of textiles with madder root a potential risk for dye house workers and consumers must be assumed. Following the precautionary principle it is recommended that extracts of *Rubia tinctorum* should not be used for textile dyeing any more.

Acknowledgement

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List of figures and tables

Figure 1: Madder root dyeing process, sample taking and sample code

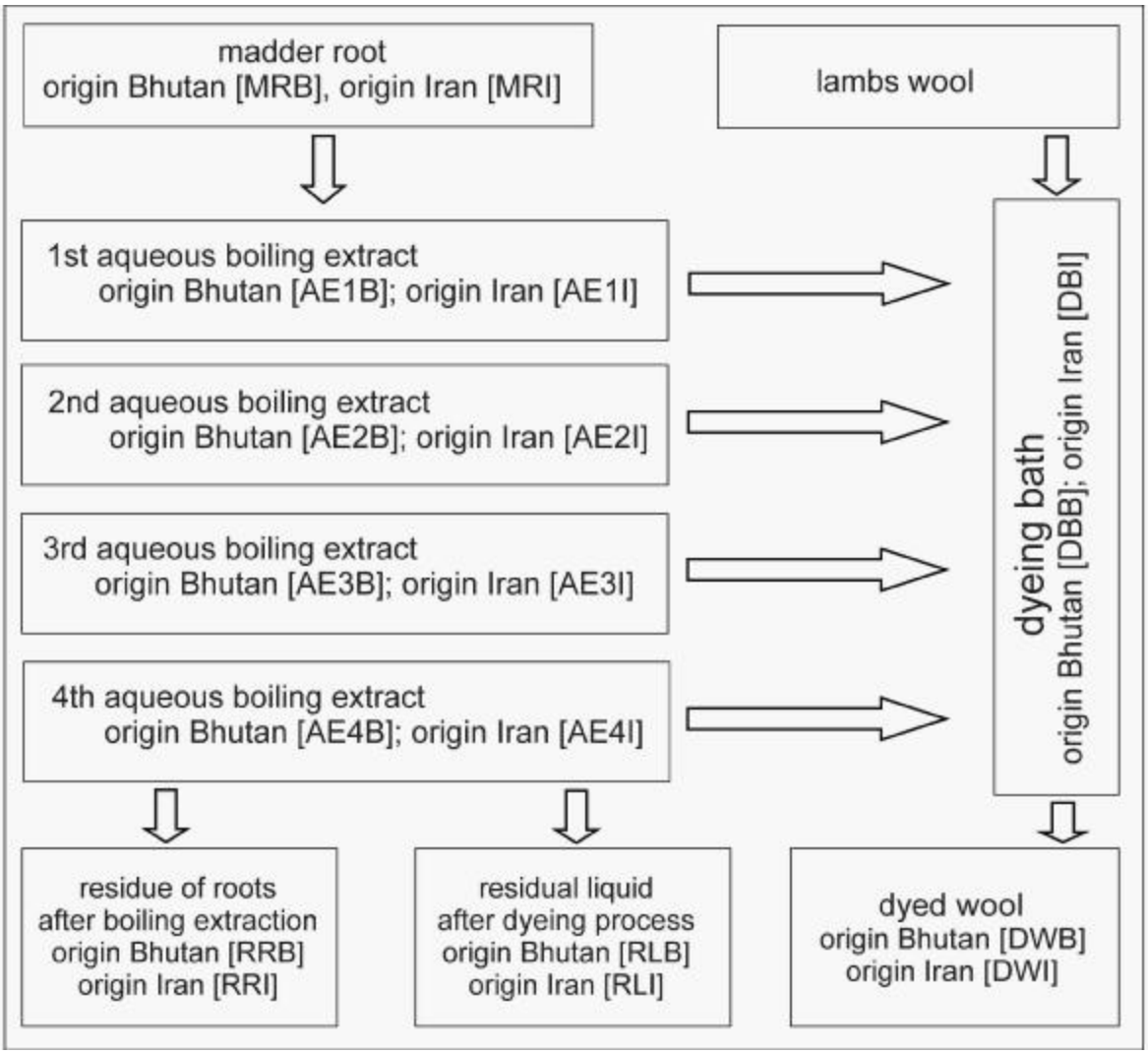


Figure 2: HPLC chromatogram of madder root (MR), dyeing bath (DB) and wool (DW) extracts from origin Iran (I) and Bhutan (B)

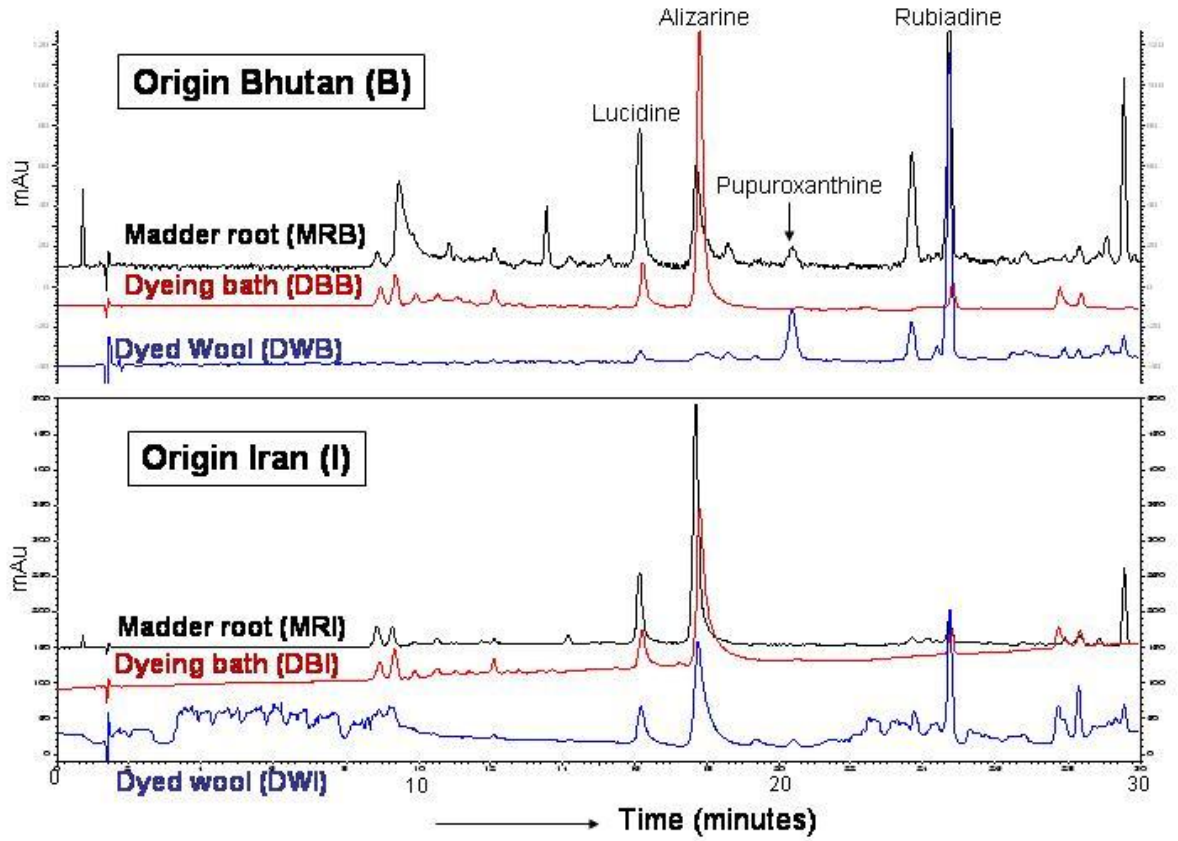
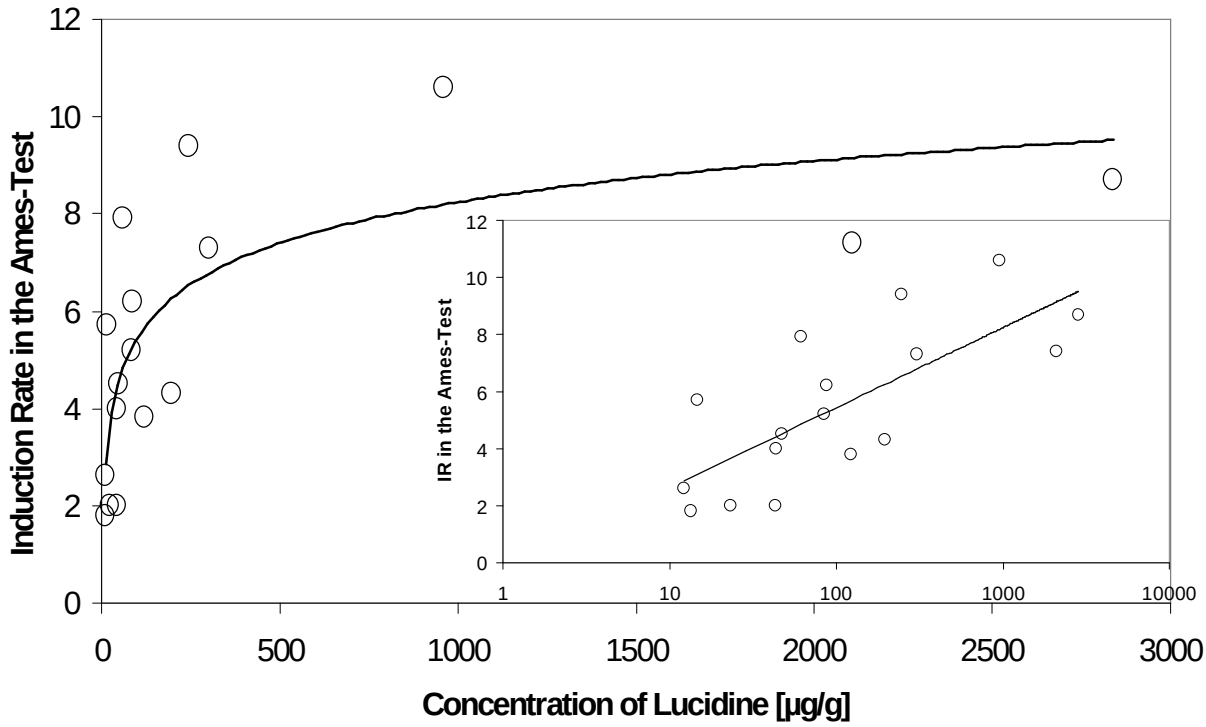


Figure 3: Correlation of lucidine concentration in samples from different stages of the dyeing process with the induction rate (IR) in *S. typhimurium* strain TA100. The insert is a semi-logarythmic plot of the slope of the curve.



	TA98 -S9			TA98 +S9			TA100 -S9			TA100 +S9			TA1537 -S9			TA 1537 +S9		
	mean n=3	RSD [%]	IR	mean n=3	RSD [%]	IR	mean n=3	RSD [%]	IR	mean n=3	RSD [%]	IR	mean n=3	RSD [%]	IR	mean n=3	RSD [%]	IR
NC (H_2O)	21	24		24	24		109	8		99	7		5	0		6	44	
DBI 1ml	**56***	26	**2,7**	39	26	1,6	**792**	9	**7,3**	**530**	3	**5,4**	**31**	9	**6,2**	**28**	33	**4,7**
DBB 1ml	1 T	149	**-**	**67**	5	**2,8**	55 T	19	0,5	**736**	12	**7,4**	**38** t	14	0,1	**49**	24	**8,2**
PC	252	18	**12,0**	381	5	**15,9**	654	12	**6,0**	717	10	**7,2**	502	59	**100,4**	59	12	**9,8**

Table 1: Results of the Ames test with the aqueous dyeing baths (DBI and DBB)

NC: Negative Control (Water)

PC: Positive Control (TA98-: Nitrofluorene 1,5 µg/plate; TA100-: Sodium azide 0,5 µg/plate, TA1537-: 9-Aminoacridene 50 µg/plate; TA98+: 2-Aminoanthracene 2,0 µg/plate, TA100+ and TA1537+: 2-Aminoantracene 2,5 µg/plate)

RSD: Relative Standard Deviation [%]

IR: Induction Rate (number of revertants of the sample / number of revertants of the NC)

T: strong toxic effect, t: low toxic effect (IR sample < IR NC)

n=3 indicates three replicates/plates of the sample

bold letters indicate mutagenic samples

	TA98 -S9			TA98 +S9			TA100 -S9			TA100 +S9		
	mean n=3	RSD [%]	IR	mean n=3	RSD [%]	IR	mean n=3	RSD [%]	IR	mean n=3	RSD [%]	IR
NC (DMSO)	19	26		24	20		119	8		103	8	
DBB	**40**	10	**2,1**	41	8	1,7	**454**	19	**3,8**	200	7	1,9
DBI	32 T	49	**-**	43	21	1,8	**682**	17	**5,7**	**453**	7	**4,4**
PC	252	18	**13,3**	381	5	15,9	654	12	**5,5**	717	10	**7,0**
NC (DMSO)	19	39		31	43		93	8		86	16	
AE1B	26	21	1,4	31	24	1,0	**488**	2	**5,2**	**218**	11	**2,5**
AE1I	30 T	11	1,5	38	15	1,2	**739**	13	**7,9**	**343**	10	**4,0**
AE2B	25 t	12	1,3	35	16	1,1	**401** t	6	**4,3**	**226**	7	**2,6**
AE2I	**68** T	19	**3,5**	49	36	1,6	**992**	4	**10,6**	**630**	14	**7,4**
AE3B	16	30	0,8	37	11	1,2	184	8	**2,0**	106	11	1,2
AE3I	**41** T	14	**2,1**	30	22	1,0	**879** t	15	**9,4**	**324**	4	**3,8**
AE4B	15 t	25	0,8	30	15	1,0	182	10	**2,0**	134	8	1,6
AE4I	19 t	32	1,0	27	21	0,9	**379**	6	**4,1**	145	3	1,7
PC	242	9	**12,5**	202	6	**6,5**	632	21	**6,8**	452	7	5,3

Table 2: Results of the Ames test with the organic extracts (ethyl acetate) of the dyeing baths (DBI and DBB) and of the aqueous boiling root extracts (AE 1-4)

NC: Negative Control (Water)

PC: Positive Control (TA98-: Nitrofluorene 1,5 µg/plate; TA100-: Sodium azide 0,5 µg/plate, TA98+: 2-Aminoanthracene 2,0 µg/plate, TA100+: 2-Aminoantracene 2,5 µg/plate)

RSD: Relative Standard Deviation [%]

IR: Induction Rate (number of revertants of the sample / number of revertants of the control)

20 µl DMSO extract per plate applied corresponding to 0.3 – 0.4 ml aqueous extracts or dyeing baths

T: strong toxic effect, t: low toxic effect (IR sample < IR NC)

n=3 indicates three replicates/plates of the sample

bold letters indicate mutagenic samples

	TA100 -S9			TA100 +S9		
	mean n=3	RSD [%]	IR	mean n=3	RSD [%]	IR
NC (DMSO)	98	9		88	5	
MRB EtOH 20µl	**263 t**	6	**2,7**	**654**	11	**7,4**
MRB EtOH 40µl	**258 T**	26	**2,6**	**547**	12	**6,2**
MRB EtOAc 20µl	**319**	5	**3,3**	**499**	8	**5,7**
MRB EtOAc 40µl	**503 T**	6	**5,1**	**535**	8	**6,1**
MRI EtOH 20µl	0 T	-	**-**	**762**	8	**8,7**
MRI EtOH 40µl	0 T	-	**-**	n.a.	-	-
MRI EtOAc 20µl	**1327**	10	**13,5**	**922**	13	**10,5**
MRI EtOAc 40µl	0 T	-	**-**	**1224**	12	**13,9**
RRB EtOH 20µl	155	6	**1,6**	**564**	6	**6,4**
RRB EtOH 40µl	**206**	2	**2,1**	**638**	5	**7,3**
RRB EtOAc 20µl	**232**	9	**2,4**	**381**	4	**4,3**
RRB EtOAc 40µl	**337**	12	**3,4**	**380**	8	**4,3**
RRI EtOH 20µl	195	7	**2,0**	**775**	3	**8,8**
RRI EtOH 40µl	**296**	6	**3,0**	**753**	11	**8,6**
RRI EtOAc 20µl	**330**	4	**3,4**	**558**	5	**6,3**
RRI EtOAc 40µl	**495**	3	**5,1**	**692**	7	**7,9**
RLB EtOAc 20µl	148	4	1,5	102	7	1,2
RLB EtOAc 40µl	179	6	1,8	117	7	1,3
RLI EtOAc 20µl	**395**	6	**4,0**	136	11	1,5
RLI EtOAc 40 µl	**609**	4	**6,2**	**198**	13	**2,3**
PC	730	13	**7,4**	392	7	**4,5**

Table 3: Results of the Ames test with the organic extracts (ethyl acetate EtOAc, and ethyl alcohol EtOH) of the madder roots (MR), of the residues of the madder roots after extraction (RR) and of the residual liquid after dyeing process (RL)
NC: Negative Control (Water)
PC: Positive Control (TA100-: Sodium azide 0,5 µg/plate, TA100+: 2-Aminoantracene 2,5 µg/plate)
RSD: Relative Standard Deviation [%]
IR: Induction Rate (number of revertants of the sample / number of revertants of the control)
20 µl, 40 µl resp. DMSO extract per plate applied corresponding to 20 mg, 40 mg resp. dry matter of the roots, 16-21 mg, 32-42 mg resp. of the root residues and 0,4, 0.7-0.8 ml of the residual liquid
T: strong toxic effect, t: low toxic effect (IR sample < IR NC)
n=3 indicates three replicates/plates of the sample
bold letters indicate mutagenic samples

	TA100 -S9			TA100 +S9		
	mean n=3	RSD [%]	IR	mean n=3	RSD [%]	IR
NC (DMSO)	98	9		88	5	
DWB EtOAc 20 µl	131	14	1,3	134	10	1,5
DWB EtOAc 40 µl	167	11	1,7	**229**	15	**2,6**
DWI EtOAc 20 µl	**339**	4	**3,5**	**207**	13	**2,4**
DWI EtOAc 40 µl	**440**	4	**4,5**	**268**	8	**3,0**
PC	730	13	**7,4**	392	7	**4,5**
NC (AS-5.5)	76	29		110	8	
DWB AS-5.5 500µl	93	13	1,2	87	2	0,8
DWB AS-5.5 1,000µl	85	18	1,1	111	10	1,0
DWI AS-5.5 500µl	103	18	1,4	119	14	1,1
DWI AS-5.5 1,000µl	114	2	1,5	134	6	1,2
PC	593	7	**5,7**	381	6	**3,5**
NC (AS-8)	88	16		115	8	
DWB AS-8 500µl	73	15	0,8	112	1	1,0
DWB AS-8 1,000µl	129	30	1,5	144	20	1,3
DWI AS-8 500µl	**178**	3	**2,0**	122	1	1,1
DWI AS-8 1,000µl	**285**	17	**3,2**	145	10	1,3
PC	593	7	**5,7**	381	6	**3,5**
NC (tw)	104	10		110	9	
DWB tw 500µl	141	35	1,4	100	10	0,9
DWB tw 1,000µl	111	13	1,1	87	9	0,8
DWI tw 500µl	131	13	1,3	112	12	1,0
DWI tw 1,000µl	160	8	1,5	105	19	1,0
PC	593	7	**5,7**	381	6	**3,5**

Table 4: Results of the Ames test with the organic extracts (ethyl acetate EtOAc), the artificial acid (pH 5.5) sweat extracts (AS-5.5), the artificial alkaline (pH 8) sweat extracts (AS-8) and the tap water extracts (tw) of the dyed wool.
NC: Negative Control (Water)
PC: Positive Control (TA100-: Sodium azide 0,5 µg/plate, TA100+: 2-Aminoantracene 2,5 µg/plate)
RSD: Relative Standard Deviation [%]
IR: Induction Rate (number of revertants of the sample / number of revertants of the control)
20 µl, 40 µl resp. DMSO extract per plate applied corresponding to 100 mg, 200 mg resp. dry matter of the dyed wool
500 µl, 1,000 µl resp. sweat extract per plate applied corresponding to 16.5, 33 mg dry matter of the wool
n=3 indicates three replicates/plates of the sample
bold letters indicate mutagenic samples

	AQ concentration [µg/ml]			
sample	lucidine	alizarine	pupuroxanthine	rubiadine
MRI	2840	8168	98	129
MRB	2106	1715	76	1314
DBI	15	68	n.d.	3
DBB	122	565	11	165
DWI	47	151	n.d.	42
DWB	12	6	13	126

Table 5: Concentrations of the anthraquinones (AQ) in the organic (ethyl acetate) madder root (MR), dyeing bath (DB) and dyed wool (DW) extracts from the origin Iran (I) and Bhutan (B) n.d.: concentration below detection limit.