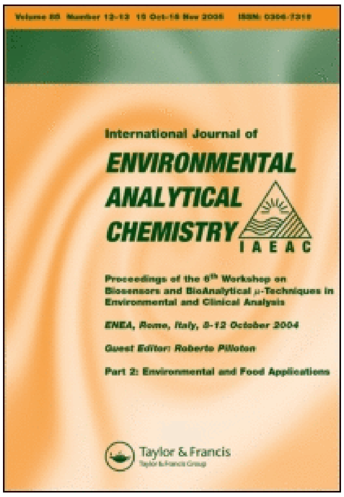

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CHEMICAL CHARACTERIZATION OF SILAGE EFFLUENTS AND THEIR INFLUENCE ON SOIL BOUND HEAVY METALS

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Silage effluents, generated during silaging of various crops, are produced in high amounts in cattle breeding farms. Due to their acidity and high content of dissolved organic matter, they are causing disposal problems. On the other hand, their ability to mobilise soil bound trace elements (i.e. heavy metals) might be useful for the decontamination of polluted soils.

With an aim to perform metal leaching studies, silage effluents from various ensiled crops (maize, rape, grass, clover, sugar-beet leaves) were collected from Bavarian farms and analysed for their main inorganic and organic constituents. Important TOC sources (TOC concentrations from 13.9 to 53.6 gl^{-1}) are short chain aliphatic acids (concentrations between 235 and 638 $mM \cdot l^{-1}$; predominant compound: lactic acid), amino acids (ranging from 22.8 to 151 $mM \cdot l^{-1}$) and polypeptides (concentrations from 3.8 to 20.0 $g \cdot l^{-1}$).

The release of heavy metals from two adsorbents (bentonite and peat) and from a polluted soil under the influence of silage effluents was studied in batch tests. The leaching efficiencies for the soil bound metals increase following the order: sugar-beet leaves < clover < maize < rape < grass.The extraction rates obtained with grass silage juice are: Cd 74.7%, Zn 55.7%, Cu 53.5%, Ni 38.9%, Cr 12.7% and Pb 8.9%. After neutralisation the leaching rates dropped, with the exception of copper.

KEY WORDS: Silage effluents, heavy metals, soils, leaching, amino acids, lactic acid.

INTRODUCTION

Effluents are released in large amounts during silaging of crops. Both the volume and chemical composition of the effluents depend on many factors related to the ensiled crop, the silaging conditions and the process time. Mason[1] for instance estimated a total volume of about 1.5 billion litres of effluents produced in 1987 in England mainly by silaging of grass. Despite the great variability in the chemical composition of the effluents, the main constituents are inorganic ions (nitrate, chloride, phosphate, ammonia, alkaline and alkaline earth ions, iron, manganese and trace elements) and organic compounds, such as amino acids, polypeptides, aliphatic and aromatic acids, carbohydrates and alcohols. Due to these constituents, silage effluents can be used as nutrient suppliments for animal feeding as well as for the fertilization of soils[2–5]. Nevertheless the composition of the effluents provides the potential for environmental contamination, if unconditioned silage juices are improperly spread onto soils or discharged into sewers and surface waters. Possible harmful effects are the poisoning of fish, oxygen depletion and eutrophication of surface waters, overdressing of soils, alterations to plant availability and mobility of micronutrients and trace elements in soils and the transfer of heavy metals together with organic matter into groundwaters[6–9]. Therefore Merriman[10] stated that silage effluent "is the most polluting waste produced on farms". This statement is derived from his summary of pollution incidents caused by silage effluents.

With regard to their content of metal complexing and chelating compounds, silage effluents should be considered as possible extractants for the sanitation of polluted soils by leaching methods. This might offer the benefit of an ecologically tolerable way both for the decontamination of soils and the treatment/disposal of silage effluents.

Therefore it has to be tested whether silage effluents are able to replace synthetic chelating agents (i.e. NTA, EDTA) or technically manufactured naturally occurrig compounds like citric acid within remediation activities. This required a detailed investigation of factors which determine the interactions between silage effluents and soil bound heavy metals. Consequently effluents from various ensiled crops were collected from several Bavarian farms. Physico-chemical parameters as well as the main organic and inorganic constituents were analysed. Leaching characteristics of silage juices were evaluated in batch experiments using soil components (bentonite and peat), to which heavy metals had been added (target concentrations: 2 and 20 times higher than the guideline values for sewage sludge application onto soils, established by the German sewage sludge degree "KSV") and a soil polluted by a long-term treatment with sewage sludge. The test scheme has been described in this journal previously[11].

In this presentation, findings of our experimental work are reported with special emphasis on silage effluent analysis.

MATERIALS

Soil components and soil sample

Bentonite (Schwaiba Comp., Germany), a smectite- (esp. montmorillonite) rich mud stone. Cation Exchange Capacity (CEC): 392.5 mmol$_c$·kg^{-1}, pH (0.01 M $CaCl_2$): 7.54. *Peat*, sampled from the upper 30 cm of a Bavarian fen soil, dried at 60°C, 0.56 mm sieved. Organic matter content: 49%, CEC: 827 mmol$_c$·kg^{-1}, carbonate content: 2.4%, pH ($CaCl_2$): 5.46.

Soil: a sandy silty loam, polluted by a long-term treatment with sewage sludge, air-dried, 0.56 mm sieved. pH ($CaCl_2$): 6.6, CEC: 342 mmol$_c$·kg^{-1}, organic matter: 10.3%, carbonate content: 13.4%, metal content (mg·kg^{-1}): Cd 46.1, Cr 246, Cu 327, Hg 3.9, Ni 85.4, Pb 1328 and Zn 1646.

Silage effluents

Effluents from five ensiled crops were collected in amounts of several litres from Bavarian farms during 1991 and 1992. The time span between the silaging of the crops and the sampling of the silage effluents ranged from fourteen days to three months. Prior to analysis or experimental application, the samples were stored in completely filled PE screw top tubes or in tubes with a headspace of nitrogen and refrigerated at 4°C.

According to results published by Woll-Schaaf *et al.*[12], investigations confirmed the stability of the main organic components under these storage conditions. Nevertheless transformations of minor compounds during the storage period can not be excluded.

Metal salts

Analytical grade divalent acetates of Cu, Cd, Ni and Pb, purchased from Merck Comp., Darmstadt (Germany).

ANALYTICAL METHODS AND EQUIPMENT

The dry matter content was determined gravimetrically after drying at 105°C. The total organic carbon (TOC) content was analysed by a Rosemount-Dohrmann DC-180 TOC-analyzer using a combined UV-persulfate sample digestion method. Protein analysis was performed according to Lowry *et al.*[13]. Elements were quantified by flame and graphite furnace AAS (Perkin Elmer) and ICP-AES (Jobin Yvon JY 70+). Solid materials were digested with aqua regia prior to analysis. Inorganic anions and ammonia were measured by means of segmented continous flow analysis and photometric detection (Skalar). Short chain aliphatic acids were identified with a DX 300 ion chromatograph (Dionex), equipped with an Ion Pac ICE-As5 ion exclusion separation column, AMMS-ICE suppressor column and a conductivity detector[14]. Diluted perfluorobutyric acid, purchased from Fluka, served as eluent. Amino acids were separated after derivatization with o-phthaldialdehyde in a HPLC system (LKB and Knauer) using a Spherisorb ODS-2 C_{18} column and a sodium phosphate/acetonitrile eluent gradient[15]. The detection was performed with a F-1000 fluorescence detector (Hitachi). Medium and high lipophilic aliphatic and aromatic acids were transformed to their methylesters, extracted with n-hexane and analysed by GC-MS (GC 5890 II and MD 5970, both Hewlett Packard, SP-2380 [biscyanopropylsiloxane] column, Supelco).

Experimental procedures

The adsorption of heavy metals on bentonite and peat as well as the leaching of metals from contaminated materials including soil were carried out in centrifuge tubes clamped into a rotating apparatus. The solid: liquid ratio was 1 : 10. To prevent percipitation of metals during the adsorption process, the suspensions were buffered at pH 4.6–4.8 (acetate buffer). At the end of a shaking period of 18 to 24 hours the adsorbents were separated from the suspensions by centrifugation and subsequent 0.1 μm filtration (cellulose nitrate membrane) of the supernatant.

The extraction tests were performed as duplicates with 1.0 g of sorbents or soil, suspended in 10 ml of silage juices and rotated during 24 hours with 12 rpm. Additionally some long-term tests with a duration of 9 days were conducted. Depending on test conditions, the reaction pH was either not regulated or adjusted to a pH value of 4.15 or 7.0 by the addition of mineral acids or bases. During the test the pH value was measured several times and adjusted if necessary.

The mass balance of the metals was calculated from the differences between their initial and final concentrations in the liquid phase. For a critical evaluation of the results, the metal content of the contaminated and extracted materials was determined several times after digestion with aqua regia.

RESULTS

Silage effluent analysis

Information about basic chemical and physical properties of silage effluents is provided in Table 1.

The pH values of the effluents range typically from 4.1 to 4.5. Only the clover seepage water has a higher pH (5.1). The high sodium hydroxide demand for the neutralization of 11 of silage effluent (mean: 206 ml lm NaOH) reflects the concentration of proton donating compounds. The highest and the lowest TOC values differ by a factor of four. The mean TOC content is 34.0 $g \cdot l^{-1}$. The TOC concentration, dry matter content and the NaOH demand of the effluents are closely related: The highest and lowest values are jointly recorded for grass juice 1 and maize juice 2, respectively.

Chloride and phosphate are the predominant inorganic anions in silage juices (Table 2). Nitrite is present in traces generally, whereas the concentration of nitrate varies within four orders of magnitude. The remarkable differences in the nitrate contents of the grass juices can not be explained. Because of sample ageing, a repeat analysis was not possible. As a comparison of the ammonia, nitrate and nitrite concentrations reveals, inorganic

Table 1 Chemical and physical properties of silage effluents.

Silage juice	*Grass*		*Clover*	*Maize*		*Rape*		*Sugar-beet l.*
	G1	G2	C	M1	M2	R1	R2	S
Colour	yellow-orange	yellow-orange	yellow-orange	yellow-green	brown-orange	yellow	yellow	yellow-green
pH	4.1	4.3	5.1	4.0	4.4	4.4	4.2	4.4
Cond [$mS \cdot cm^{-1}$]	n.m.	n.m.	16.37	11.82	n.m.	14.39	18.57	17.69
NaOH 1m [ml]	343	302	148	279	113	113	185	168
Dry matter [$g \cdot l^{-1}$]	115	85.3	57.2	58.7	32.7	35.6	55.2	55.6
TOC [$g \cdot l^{-1}$]	53.57	40.93	39.38	36.44	13.88	27.41	29.88	30.13
Protein [$g \cdot l^{-1}$]	20.0	n.m.	7.5	8.3	n.m.	5.5	9.3	3.8

Legend: NaOH 1 n: amount of 1 n NaOH for neutralization of 1 L of silage effluent
cond: conductance n.m.: not measured

Table 2 Anions, ammonia and sulfur in silage effluents.

Silage juice	*G1*	*G2*	*C*	*M1*	*M2*	*R1*	*R2*	*S*
				concentration [$mg \cdot l^{-1}$]				
Ammonia	1065	1520	1173	462	476	429	209	546
Chloride	4077	6386	1266	1083	892	1197	2325	3726
Nitrite	0.91	< 0.1	0.42	0.47	< 0.1	0.18	0.26	0.19
Nitrate	1854	< 1	5.19	8.94	8.80	81.0	128	64
Phosphate	4620	3400	1641	2463	978	1311	510	1431
Sulfur	716	548	165	164	436	369	183	n.m.

n.m.: not measured

Table 3 Metal content of untreated silage effluents.

Silage juice	*G1*	*G2*	*C*	*M1*	*M2*	*R1*	*R2*	*S*
Metals	Concentration [$\mathrm{mg \cdot l^{-1}}$]							
Cd	0.117	< 0.026	0.037	0.027	0.055	0.014	0.036	0.059
Cr	0.404	0.539	0.093	0.075	0.130	0.061	0.064	0.173
Cu	0.759	< 0.139	0.268	0.175	0.148	0.530	0.055	0.104
Ni	0.338	0.393	0.346	0.034	< 0.075	0.087	0.033	0.072
Pb	0.192	0.591	0.133	0.078	< 0.512	0.061	0.047	0.393
Zn	14.8	12.8	10.2	25.7	2.20	1.86	3.85	1.94
Al	75.0	41.9	26.4	4.03	7.42	16.5	4.68	116
Fe	327	56.6	211	149	58.7	77.7	27.9	297
Mn	96.7	14.7	26.5	9.59	4.12	8.92	10.2	12.3
Ca	2695	4114	2146	1226	1531	1773	2488	39.8
Mg	1113	1087	562	557	148	170	516	607

Annotation: Differences in the metal-specific determination limits due to different sample dilutions prior to analysis.

Table 4 Metal content of neutralized silage effluents.

Silage juice	*G1*	*G2*	*C*	*M1*	*M2*	*R1*	*R2*	*S*
Metals	Concentration [$\mathrm{mg \cdot l^{-1}}$]							
Cd	0.020	< 0.028	< 0.014	< 0.007	0.058	0.009	< 0.014	< 0.013
Cr	0.219	0.233	0.015	0.041	< 0.132	0.042	0.032	0.098
Cu	0.106	0.145	0.259	0.216	< 0.142	0.681	0.031	0.127
Ni	0.346	0.473	0.347	0.027	< 0.087	0.065	0.057	0.064
Pb	0.093	< 0.550	< 0.054	< 0.068	0.594	< 0.037	< 0.052	0.211
Zn	3.93	2.18	5.15	5.82	1.25	1.44	3.28	1.71
Al	3.14	4.27	0.338	0.625	2.36	0.754	0.251	26.3
Fe	56.3	67.2	3.85	3.49	9.42	7.93	4.29	50.5
Mn	6.51	1.24	8.10	0.882	0.479	1.55	2.42	15.3
Ca	1190	1741	1932	607	1022	1434	2357	36.0
Mg	917	700	568	424	147	163	504	607

Annotation: Differences in the metal-specific determination limits due to different sample dilutions prior to analysis.

nitrogen is present mainly in the form of ammonia. The sulfur data essentially represents the sulfate concentration. The highest ion and sulfur concentrations are always found in the grass silage effluents.

The concentration of those heavy metals which are considered as potential soil pollutants is always below 1.0 $\mathrm{mg \cdot l^{-1}}$, with the exception of zinc (Tables 3 and 4). The concentration of the individual metal ions increases following the order: Cd < Ni ≈ Cr < Pb < Cu < Zn < Mn < Al < Fe < Mg < Ca. Assuming a significant fraction of the metals is present in organically complexed form, the overall complexation capacity of the effluents, which is an important factor for the mobilization of soil bound metals, is lowered by a proportional binding of potential ligands.

The neutralization of the effluents with a concentrated sodium hydroxide solution reduces the concentration of most metals, probably because of the precipitation or coprecipitation of metal hydroxides, phosphates or carbonates. The highest relative concentration decreases are noticeable for aluminium (mean reduction factor: 7.7), iron

Table 5 Quantification of selected amino acids.

Silage juice	*G1*	*C*	*M1*	*R1*	*R2*	*S*
Amino acids			Concentration [mM·l^{-1}]			
Alanine	35.38	31.48	9.67	12.86	25.16	8.87
Arginine	1.42	n.d.	0.05	n.d.	n.d.	n.d.
Asparatic acid	13.82	2.47	2.82	1.10	0.57	0.19
Glutamic acid	3.98	10.30	3.86	2.67	0.27	4.70
Glycine	14.81	10.19	4.49	4.17	8.27	2.10
Histidine	0.55	1.96	0.09	1.25	2.55	0.76
Isoleucine	10.36	7.86	2.35	2.16	4.96	1.39
Leucine	17.71	11.17	4.52	3.19	8.51	1.71
Methionine	3.35	1.73	0.58	0.66	1.89	0.29
Phenylalanine	7.94	4.59	1.55	1.76	4.38	0.64
Serine	11.85	0.61	0.66	0.57	3.75	0.26
Threonine	12.93	7.43	2.03	1.15	4.00	n.m.
Tyrosine	1.89	0.19	0.16	0.27	0.23	0.14
Valine	14.97	11.69	3.92	3.93	7.74	1.79
Σ	**150.96**	**101.67**	**36.75**	**35.74**	**72.28**	**22.84**

n.d.: not detectable

(5.9) and manganese (5.0). The neutralization has no or only slight effects on the solubilities of nickel and copper.

Due to the detection method, only primary amino acids could be detected (Table 5). The amino acid content ranges from 22.8 mM·l^{-1} to 151 mM·l^{-1}. Main components are alanine, glycine, leucine and valine. Between 54.9% (grass 1) and 68.7% (rape 2) of the total amino acid content of the silage effluents is attributed to these four acids. Glutamic acid accounts for 10% of the amino acid content of the clover juice and for 20% of the amino acid fraction of the sugar-beet leaves effluent. The amino acid content of the effluent decreases following the order: grass 1 > clover > rape 2 > maize 1 ≈ rape 1 > sugar-beet leaves.

Whereas the maize 1 and rape 1 juices are very similar both with respect to their total amino acid content and to the relative importance of the specific compounds, they differ significantly with regard to their aliphatic acid composition (Table 6).

Eight aliphatic acids could be identified and quantified in silage juices. Due to the

Table 6 Quantification of short-chain aliphatic acids.

Silage juice	*G1*	*C*	*M1*	*M2*	*R1*	*R2*	*S*
Acids				Concentration [mM·l^{-1}]			
Formic	1.02	6.08	0.72	1.22	1.09	0.52	0.35
Acetic	95.17	152.81	102.56	37.42	41.47	68.53	107.08
Propionic	3.70	6.40	0.30	1.24	1.56	1.26	3.21
Pyruvic	1.91	3.59	1.08	0.57	0.44	0.37	9.85
Lactic	519.25	172.34	286.78	188.39	214.09	359.57	255.50
Glyceric	1.70	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Succinic	14.56	33.11	9.29	6.20	4.33	4.66	4.87
Isocitric	0.70	0.26	0.78	n.d.	0.16	0.41	0.13
Σ	**638.0**	**374.59**	**401.51**	**235.04**	**263.14**	**435.32**	**380.99**

n.d.: not detectable

chromatographic conditions (incomplete separation from fully ionized compounds, elution within the void volume) oxalic acid could be detected in the rape 1 juice but not quantified. Six acids (lactic, acetic, succinic, propionic, pyruvic and formic) were always present, whereas iso-citric and glyceric acid were found in a major or minor number of samples, respectively.

As expected with regard to the biochemical reactions which are fundamental to the silaging process, lactic acid is the predominant compound within the group of determined aliphatic acids. The lactic acid content ranges from 172 to 519 mM·l^{-1}. Between 46.0% (clover juice) and 82.6% (rape 2) of the total aliphatic acid content (mean fraction: 72.9%) and from 15.8% (clover) to 48.9% (maize 2) of the TOC content (mean fraction: 32.9%) are attributed to lactic acid. The concentration values of acetic acid, the second most important compound, range from 37.4 to 152.8 mM·L^{-1} with TOC proportions between 3.6% (rape 1) and 9.3% (clover; mean fraction: 6.4%).

The total amounts of aliphatic and amino acids in several silage effluents are summed up and related to the TOC content in Table 7. The contribution of aliphatic acids to the TOC content is relatively constant with a mean of 39.4%. The amino acid fraction of the TOC varies significantly (from 3.8% to 14.8%), the mean amounts to 9.6%. The mean molar concentration of aliphatic acids in silage effluents is about six times higher than the mean of the amino acids. Between one and two thirds of the organic matter content of silage effluents could be identified as aliphatic and amino acids.

Aromatic, alicyclic and more hydrophobic aliphatic acids were investigated by means of GC-MS (Table 8). A total of twenty substances could be detected. Of these, eighteen compounds were completely identified with the exception of the optical configuration and two with partial details of the basic structural units of their isomers. The majority of the compounds belongs to monocarboxylic aliphatic acids. Five aromatic acids and four aliphatic dicarboxylic acids were detected. The grass juice exhibits the broadest spectrum and the highest concentrations. For a comparison of the substance concentrations in the effluents, peak area ratios were calculated as percentages of the highest found specific area. The comparison of a concentration sequence of succinic acid, derived from peak area ratios of the GC-MS chromatograms with the one assembled on the basis of the IC data reveals that this procedure provides reasonable results. The GC-MS chromatogram of the grass juice 1 is shown in Figure 1.

Metal leaching

The leaching efficiencies of grass silage juice at acidic and neutral pH conditions for heavy metals, bound on bentonite or peat, are summarized in Figure 2. The determined

Table 7 TOC-identification of silage effluents.

Silage juice	*Aliphatic acids*		*Amino acids*			
	mM·l^{-1} (a)	% TOC	mM·l^{-1} (b)	% TOC	molar ratio (a : b)	Σ % TOC
G1	638.0	41.1	151.0	14.8	4.2	55.9
C	374.6	30.2	101.7	13.5	3.7	34.7
M1	401.5	36.6	36.8	5.2	10.9	41.8
R1	263.0	32.8	35.7	6.6	7.4	39.4
R2	435.3	54.4	72.3	13.5	6.0	67.9
S	381.0	41.4	22.8	3.8	16.7	45.2

Table 8 Organic compounds in silage effluents identified by GC-MS.

Compounds (as methylesters)	*r[min]*	*G1*	*C*	*M1*	*R2*	*S*
			peak area ratios			
2-methylbutyric acid	8.1	8	100	83	n.d.	n.d
Iso-valeric acid	9.6	100	43	n.d.	n.d.	n.d.
Valeric acid	11.0	100	n.d.	n.d.	n.d.	n.d.
Iso-caproic acid	12.8	100	n.d.	n.d.	n.d.	n.d.
2-hydroxy-3-methylbutyric acid	13.3	47	100	n.d.	25	73
Caproic acid	13.6	100	13	n.d.	2	3
3-hexenoic acid	13.9	100	80	94	n.d.	n.d.
Malonic acid	14.3	100	8	24	n.d.	5
2-furoic acid	15.5	43	100	38	66	33
2-hydroxy-4-methylvaleric acid	15.7	100	42	33	35	24
2-hydroxy-3-methylvaleric acid	15.8	61	100	n.d.	44	31
Succinic acid	16.6	68	100	46	27	23
Cyclohexanecarboxylic acid	16.9	100	n.d.	n.d	n.d.	n.d
2-methylsuccinic acid	17.2	5	14	100	n.d.	2
Benzoic acid	17.9	32	100	9	4	5
Phenylacetic acid	19.4	100	23	27	12	4
2-hydroxybenzoic acid	19.9	14	100	n.d.	8	6
3-phenylpropionic acid	21.1	100	10	n.d.	8	4
Pimelic acid	22.2	100	27	n.d.	17	10
2-hydroxy-3-phenylpropionic acid	23.6	100	25	29	19	9

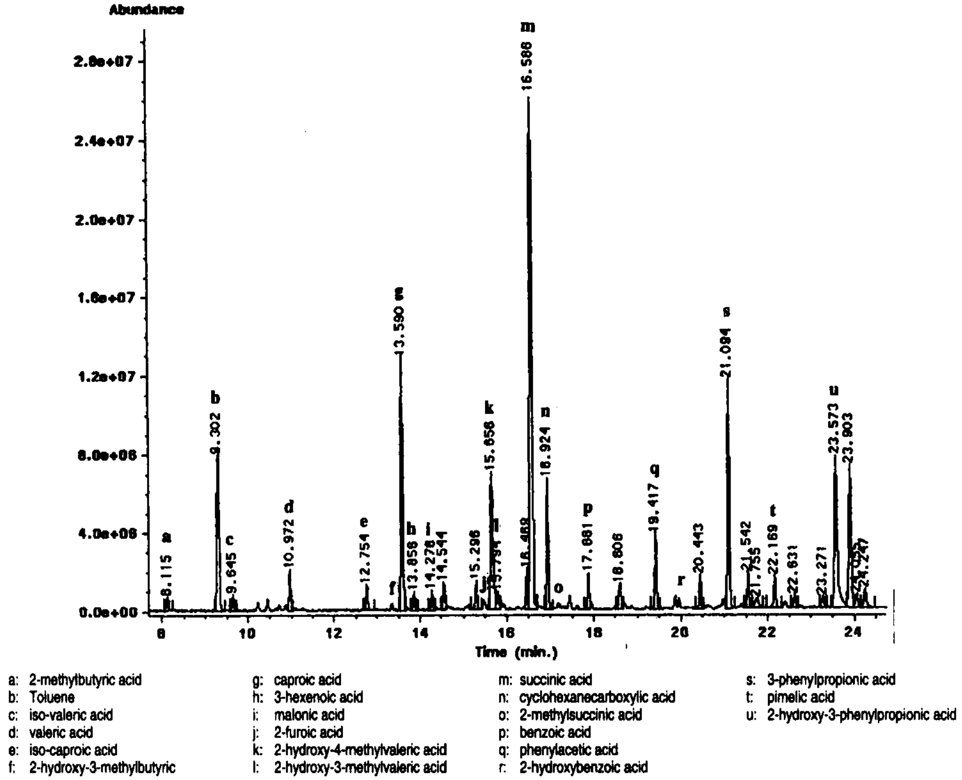

Figure 1 Total Ion GC-MS chromatogram of the n-hexane extract of grass silage juice 1 after esterification of organic acids.

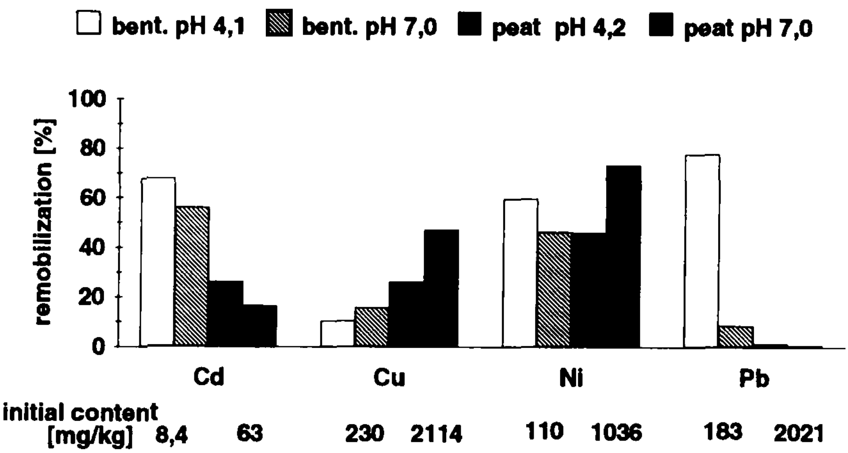

Figure 2 Remobilization of heavy metals from bentonite and peat by grass silage juice.

leaching rates reflect both metal specific interactions with the silage effluent and substrate specific sorption or complexation strengths which are influenced by the metal content. Because the metal loading of the sorbents should express different standardized pollution situations, the elimination of the influence of the sorbed metal amount on the desorption rate by adjusting equimolar metal coverings was not practicable. The pH dependency of the metal release process is clear in the case of Cd, Pb and Cu: the removal of Cd and Pb from both sorbents is favoured at acidic pH, whereas the release of Cu is elevated at neutral pH. The situation is more complicated with Ni where the effects of the metal loading and/or substrate specific binding strengths seem to overcompensate the influence of pH. From a consideration of the maximum determined leaching rates, Pb and Cu seem to be the most critical elements, because only one of four test arrangements led to a reasonable solubilization of the metals. By contrast, leaching rates greater than 40% were achieved for Ni under all experimental conditions.

The release of heavy metals from a polluted soil under the influence of various silage effluents was investigated at the orginal acidic pH (Figure 3) and at neutral pH (Figure

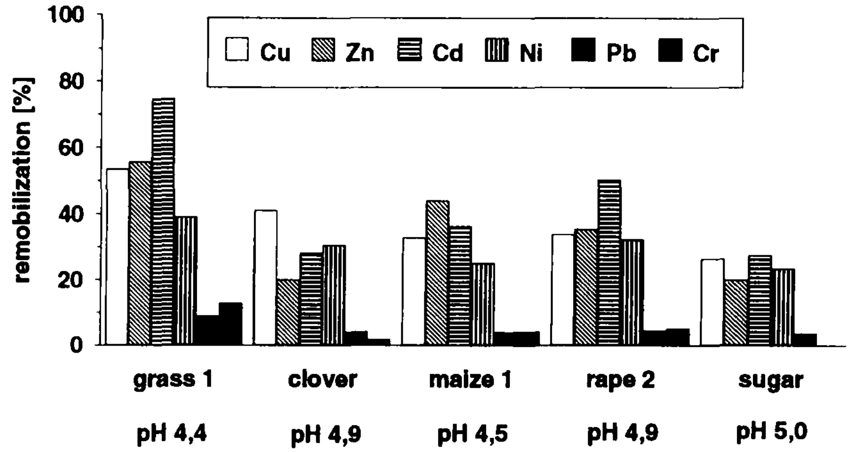

Figure 3 Remobilization of heavy metals from a polluted soil by various silage effluents at natural (acidic) pH.

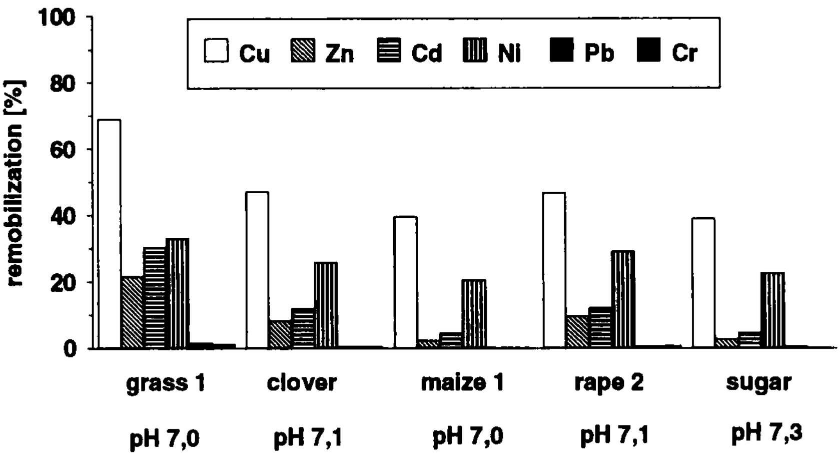

Figure 4 Remobilization of heavy metals from a polluted soil by silage effluents at neutral pH.

4). Under acidic conditions two silage effluents were able to remove more than 50% of the soil bound Cd. In general, Ni, Cu and Zn were extracted with lower efficiencies (between 20% and 55%), whereas the release of Cr and Pb typically did not exceed 10%. The mean leaching rates (in parentheses: variation coefficients) are: Cd 43.4% (45.6), Cu 37.6% (27.5), Zn 35.0% (44.2), Ni 30.3% (20.4), Cr 5.0% (92.8) and Pb 5.1% (43.0).

Within the group of silage effluents, grass juice shows the strongest influences on the metal distribution: maximum leaching rates were achieved for all metals and more than 50% of Cd, Cu and Zn were transferred into the liquid phase. The overall leaching capacities of the effluents decreased following the order: grass > rape > maize > clover > sugar beet leaves.

The neutralization of the silage effluents causes a significant change in their leaching behaviour. The extraction efficiencies for all elements are reduced with exception of Cu, whose solubility is enhanced. Next to Cu, the leachability of Ni is only slightly lowered. The mean extractability of the other elements is reduced by factors of 3.4 (Cd), 3.9 (Zn), 7.3 (Pb) and 10.0 (Cr). Therefore the leaching patterns of all silage effluents are now dominated by Cu and Ni. The mean leaching rates.at approximately neutral pH are (in parentheses: variation coefficients): Cu 48.3% (25.3), Ni 26.3% (19.2), Cd 12.7% (83.3), Zn 8.9% (88.0), Pb 0.7% (82.0) and Cr 0.5% (75.9). The decrease in the coefficients of variations of the Cu and Ni extraction signifies a more homogeneous leaching behaviour of the silage effluents at neutral pH. For the most part the increase of the coefficients of variations of the Cd and Zn extraction can be traced back to the very high leaching capacity of the grass juice. The highest loss of leaching efficiency occured in the maize juice, which is the most ineffective extractant at neutral pH.

The effect of time on the leaching of soil bound metals by grass juice at neutral pH is illustrated in Figure 5. At least two thirds of the total metal release was achieved during the first eight (Zn, Cd, Cu, Ni) or 24 (Pb) hours. Whereas the solubilization of Cr, Zn and, with restrictions, Ni proceeded during the whole time span, the approximate final concentrations of Pb and Cd in solution were reached within 24 hours. A special position has the time course of the copper release: after a period of an intensive mobilization, followed by further small increases, the copper concentration dropped below the first measured value.

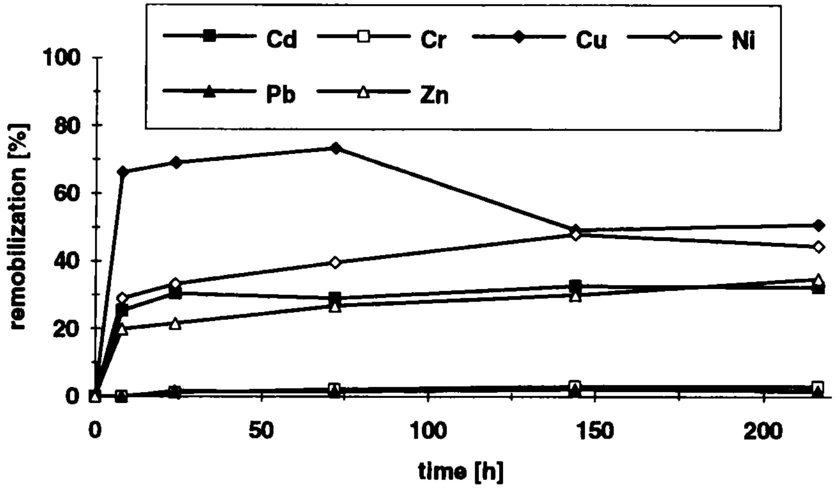

Figure 5 Kinetics of metal extraction from polluted soil by grass silage juice at neutral pH.

DISCUSSION

A comparison of the results of the silage juice analysis with those of other authors is hindered by the great variability of the silaging conditions and their diverse effects on the silage effluent composition. Side effects like the moisture content of the ensiled material or the inflow of rain water into the collection tanks can alter the concentration of the solutes. Furthermore silage juices previously investigated were generated in bench scale tests or the composition of the liquor was influenced by the supplementation of chemical preservatives or enzymes.

Hofmeister and Soeder[6] give some characteristic values of the major compounds of silage effluents: Total N: 1000 - 2000 mg·l^{-1}, total P: 500 - 1000 mg·l^{-1}, total S: ≈ 230 mg·l^{-1}, Cl^-: 3500 - 7000 mg·l^{-1} and dry matter content 50 - 75 g·l^{-1}. A comparison with these details elucidates that the grass juices tend to have substance concentrations within or exceeding the upper third of the common value ranges, whereas other effluents show relatively low concentrations of chloride (rape, maize and clover), phosphorus (rape) and sulfur (clover).

Data on the amino acid and aliphatic acid content of grass silage effluents are provided by Patterson and Walker[2]. They analysed three effluents which had received formic acid or sulphuric acid and formalin. The pH values of the liquors ranged from 3.24 to 3.82 and the dry matter content from 61.5 to 109.7 g·kg^{-1} (mean: 82.2 g·kg^{-1}). The total amino acid content varied between 152.0 and 199.2 (mean: 176.0) gram per kilogram dry matter of the liquors. A mean molar concentration of 119.8 mM·l^{-1} yields from the data transformation using an estimated factor of "1" for the density of the effluents. The concentrations of three amino acids which we have not detected or could not analyze were 10.21 mM·l^{-1} (proline), 6.31 mM·l^{-1} (lysine) and 0.73 mM·l^{-1} (cystine). Their proportion of the total amino acid amount is 14.4%. If these compounds are not included in a comparison of the total amounts, the very high amino acid content of the grass juice becomes obvious. On the other hand the relative importance of the specific acids, reported by Patterson and Walker, does not differ significantly from our results.

The same study of Patterson and Walker provides the following data on the mean

Table 9 Stability constants.

	$logK_{M_lL_l}$					
Acids	*Cd (II)*	*Cr (III)*	*Cu (II)*	*Ni (II)*	*Pb (II)*	*Zn (II)*
Lactic	1.30^a	/	2.55^a	1.64^a	1.99^a	1.86^a
Acetic	1.61^b	/	1.89^b	1.43^b	2.20^b	1.28^b
Succinic	1.47^c	6.42^b	2.93^b	/	2.40^c	2.33^c
Alanine	4.49^b	8.53^b	8.2^d	5.40^e	4.18^b	5.2^d
Glycine	4.69^a	8.41^a	8.15^a	5.78^c	4.70^c	4.96^c
Leucine	/	8.8^b	7.9^d	5.71^b	/	4.9^d
Valine	/	8.3^b	7.9^d	5.26^d	4.02^b	5.0^d

Data sources: a [18], b: [19], c: [20], d: [21], e: [22]

concentrations of aliphatic acids in grass silage juices (given in g·kg^{-1} dry matter, in parentheses: calculated molar concentrations): lactic acid 101.3 (92.4 mM·l^{-1}), acetic acid 12.27 (16.8 mM·l^{-1}) and propionic acid 1.43 (1.59 mM·l^{-1}). The stoichiometric concentration ratio of the acids is about 1 : 10 : 60 (propionic : acetic : lactic). Again the concentrations of the main components (lactic and acetic acid) of the grass juice 1 are higher (in this case: about five times) than the cited data, but the concentration ratio of the main compounds is nearly the same.

Woll-Schaaf *et al.*[12] determined the composition of seven sugar-beet leaf effluents, sampled from collection tanks. Means (in parentheses: ranges) are: pH 4.3 (4.1 – 4.8), TOC 12.4 g·l^{-1} (2.1 – 23.2), lactic acid 7.0 g·l^{-1} (1.5 – 12.4) and acetic acid 2.2 g·l^{-1} (0.7 – 3.4). The mean lactic: acetic acid ratio was 2.1 : 1 which is comparable to our findings (2.4 : 1).

A fundamental approach to explain the leaching effects of the effluents is to examine the possible interactions between aliphatic acids, amino acids and the solid bound metals. This does not mean that inorganic compounds of silage effluents, i.e. chloride, are not important. The higher complex formation constants of the organic compounds warrant more detailed consideration of these components (Table 9).

Some aspects of the metal mobilization can be discussed with reference to our former investigation of the desorption of heavy metals from bentonite and peat by 5% (w/w) aqueous solutions of glycine. The glycine concentration corresponds to a TOC content of 16 g·l^{-1} and a molar concentration of 666 mM·l^{-1} (grass juice 1: sum of aliphatic and amino acids: 789 mM·l^{-1}, lactic acid: 519 mM·l^{-1}). Differences in the test conditions are the buffering of the glycine solutions with HEPES (pH 7) and MES (pH 4.5) and the addition of an inorganic electrolyte containing 529 mg·l^{-1} of alkaline and alkaline earth salts. A direct comparison is possible for the desorption of Cd, Cu, Ni and Pb from peat at both pH values and from bentonite at neutral pH. The grass silage juice desorbs generally higher amounts of Cd, mainly higher amounts of nickel (definitely from peat), mainly lower amounts of copper (definitely from bentonite) and lower (peat) or higher (bentonite) amounts of lead than they were removed by the glycine solution. Differences have to be noted as well for the pH dependency of the reaction: metal removal by glycine is always higher at neutral than at acidic pH. As a consequence, the silage juice is more effective than glycine for the removal of heavy metals under acidic conditions at least for the mobilization from high loaded peat. This pH dependency is also applicable for the release of metals from the tested soil.

Considering the amounts of extracted metal both at acidic and neutral pH, the leaching efficiency of the silage effluents is strongly correlated with the sum of the inherent aliphatic and amino acids. The reversed leaching efficiencies of the maize and clover juice at acidic pH suggests differences in the molar ratio of aliphatic and amino acids and in the influence of pH on the leaching behaviour of these compounds. The maize juice with a higher overall leaching capacity has a lower total organic acid content than the clover juice but a higher amount of aliphatic acids. The clover juice has the highest relative amount of amino acids of all silage effluents.

There are two arguments that the relative leaching strength is related to the aliphatic: amino acid ratio. First, if the leaching behaviour of glycine is typical for amino acids (similar reactivities described for histidine[16], cysteine[17] and penicillamine[16,17]), then the contribution of the amino acid fraction to the overall leaching capacity of the effluents should decrease with decreasing pH. And second, the leachability of metals by complexing agents should correlate with the magnitude of the formation constants of the complexes formed in solution. Table 9 elucidates significant differences in the complex formation constants of aliphatic and amino acids. The complex formation constants of the amino acids surpasses that of the aliphatic acids by two to six orders of magnitude. The complexation of Cu and Ni by amino acids is favoured especially. Therefore even under acidic conditions the leaching pattern of the clover juice involves amino acid interactions with copper and nickel more clearly than the one of the other silage liquors. Despite the lower overall leaching capacity of the clover juice, its copper and nickel mobilization is higher than that of the maize juice.

These aspects are useful as well for the discussion of the leaching pattern obtained at neutral pH. The mobilization rates are the result of two contrary effects: the increasing stability of the organic complexes, especially the amino acid complexes and the decreasing solubilities of metal hydroxides, phosphates and carbonates. The enhanced activity of the amino acids can be deduced from copper solubilization which is the preferentially coordinated divalent metal: the sequence of the effluent specific leaching rates and the amino acid content agree precisely.

CONCLUSIONS

Silage effluents are liquors containing complex and diverse mixtures of organic and inorganic compounds formed and/or released during the fermentation of plant materials. TOC concentrations between 13.9 and 53.6 $g \cdot l^{-1}$ were determined in these liquids. Between one and two thirds of the total organic matter can belong to hydrophilic aliphatic acids and amino acids. Generally the concentration of aliphatic acids exceeds that of amino acids; the molar ratios range from 3.7 : 1 to 16.7 : 1. The predominant aliphatic and amino acids are lactic acid and alanine, respectively.

Depending on the amounts and molar ratios of aliphatic and amino acids, the pH conditions and the solid phase, the silage effluents were able to remove varying amounts of Cd, Cu, Zn and Ni from bentonite, peat and a polluted soil. The extractability of the metals from the soil follows the order $Cd > Zn \approx Cu > Ni >> Cr \approx Pb$ at acidic pH and $Cu > Ni > Cd > Zn > Cr \approx Pb$ at neutral pH. Some evidence suggest that the leaching behaviour of silage effluents is determined mainly by aliphatic acids at acidic pH and by amino acids at neutral pH.

A comparison of the leaching capacity of the grass juice with glycine, a moderate chelating agent (with higher complex stability constants than citric acid), reveals that in most cases, especially under acidic conditions, the application of the grass juice leads to higher metal solubilization than the use of a glycine solution. This might have practical implications for those soil remediation techniques which are based up the application of citric acid or compounds with comparable leaching properties. Considering that metal removal rates higher than 50% were reached only with silage effluents having a higher organic acid content than 0.5 $M \cdot l^{-1}$, practical examinations should be restricted to grass juices. For a more detailed investigation of the leaching capability of silage effluents, correlations between the soil binding forms of metals and their extractabilities should be studied.

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