THE FATE OF HEAVY METALS IN WASTEWATER STABILIZATION PONDS

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Abstract—The distribution of toxic heavy metals was studied throughout the process of treatment of domestic wastewater by stabilization ponds. The concentrations and distribution of free and bound zinc, cadmium, lead and copper through the various stages of a treatment plant were analyzed by Anodic Stripping Voltammetry (ASV). Only a slight decrease in the total metals concentration was observed during the various stages of the wastewater treatment plant. However, the distribution among dissolved (free and chelated) and particulate fractions did change towards solubilization of most of the particulate fraction. Within the soluble fraction a significant decrease in the free cations occurred due to a proteinaceous chelating agent(s) released by the microbial population in the ponds. A similar phenomenon was found in simulated laboratory experiments which were carried out with a *Chlorella* strain isolated from the stabilization ponds.

Key words—domestic wastewater, stabilization ponds, toxic heavy metals, *Chlorella* sp., Zn^{2+}, complexing capacity

INTRODUCTION

Effluents of wastewater treatment plants are used in Israel for irrigation of various crops according to their quality. The long term effects of heavy metals present in these effluents on soils and crops are presently unknown. Among the open questions is the fate of toxic heavy metals along the wastewater treatment path. Until recently, most of the available information on heavy metals in aquatic systems was presented as total concentrations (e.g. as analyzed by atomic absorption). However, it became more and more apparent that their chemical form (metal speciation), is of importance (Florence and Batley, 1980); it is generally accepted that it is the free ion which is the form most toxic to aquatic life (Sunda and Guillard, 1976; Anderson and Morel, 1978), and it is well established that the presence of organic compounds such as humic and fulvic acids (Greta *et al.*, 1979; Piotrowitz *et al.*, 1983), nitrilotriacetic acid (Stroveland *et al.*, 1979), and EDTA (Canterford and Canterford, 1980) in water reduces the toxicity of heavy metals, due to complexation of the free ions. Little has been reported, though, about the interaction of metals with nitrogenous organic compounds, and only some aspects of metal speciation in fresh water (Florence and Batley, 1980) and sewage (Laxen and Harrison, 1981) have been hitherto examined.

The overall objectives of the present investigation were: (a) to study the distribution of a number of

trace heavy metals (Zn, Cd, Pb and Cu) along the treatment path of domestic wastewater in stabilization ponds, and (b) to study the contribution of specific microorganisms to metal complexation capacity. The concentrations of the free cations of Zn, Cd, Pb and Cu as well as total amounts after acid digestion were determined using Anodic Stripping Voltammetry (ASV). The ASV technique (Florence and Batley, 1980; Shuman and Martin-Goldberg, 1984) permits simultaneous determination of the concentrations of several dissolved free cations in the presence of their insoluble salts and organic chelates.

When compared to conventional wastewater treatment plants based on trickling filters, activated sludge and aerated lagoons, stabilization ponds differ in several aspects which are relevant to the question of distribution of heavy metals among the various parts of this ecosystem. In contrast to the trickling filters, activated sludge and aerated lagoon processes which operate within very narrow margins of tolerance, for instance, with respect to variations in pH and redox potential, not to mention the microbial population whose stability is most critical, great variations are found even within a single stabilization pond due to the fact that light and therefore oxygen is distributed unevenly. Thus it is not uncommon to find pH values of up to 10.5 at the surface and 6.5–7.0 1 m below. Similarly, 300–400% saturation of O_2 produces a strong oxidizing environment at the surface, while anaerobic reducing conditions prevail at the bottom. As these factors might affect the solubility of metals in water, the speciation of the above mentioned cations was studied along the path of effluents from

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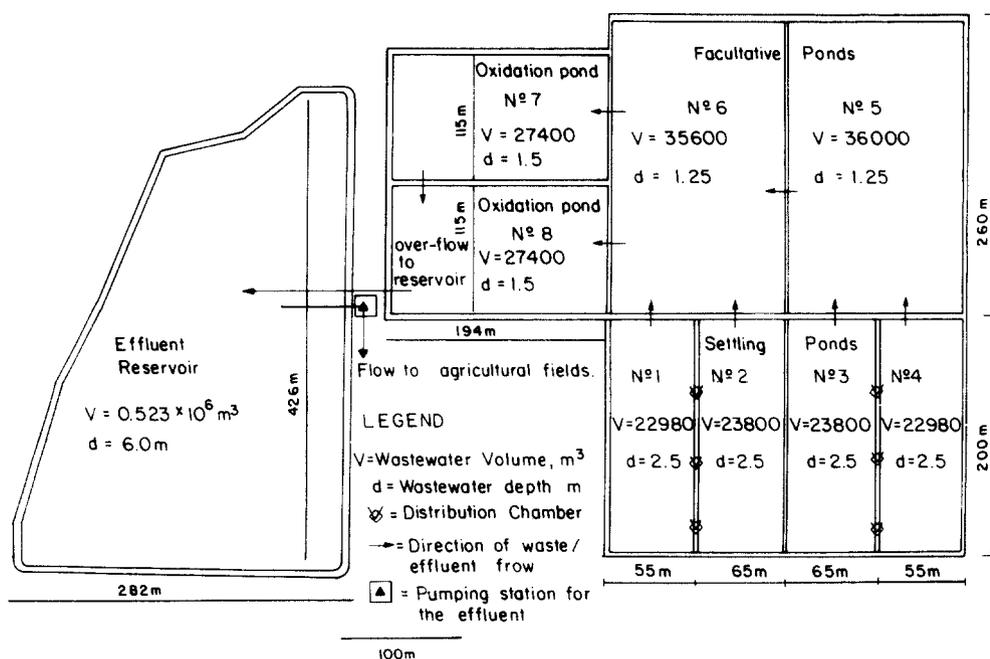*To whom correspondence should be addressed.

Fig. 1. Layout of the Beer Sheva wastewater treatment plant.

the settling ponds through the anaerobic, facultative and aerobic stages.

MATERIALS AND METHODS

Sampling site

The wastewater and effluents used for this study were taken from the Beer Sheva, Israel, domestic wastewater treatment plant (Fig. 1) which is based on stabilization ponds. The effluents are stored in a deep reservoir and utilized primarily during the summer for irrigation of cotton.

Sampling and analytical procedure

Samples from the various stages of the wastewater treatment were collected in 1 liter polyethylene containers (pre-cleaned with 10% analytical grade HNO_3 and rinsed with glass double distilled water). The samples were brought to the laboratory within 1 h, centrifuged, and immediately fractionated into filtratable and nonfiltratable fraction according to the procedure described in Fig. 2. The pellet and the nonfiltratable fraction of each sample (from 100 ml of water) were combined [designated as particulate fraction (p)] and were digested with H_2SO_4 at 450°C for 2 h or until the solutions were clear. After cooling to room temperature, pH was adjusted to 4.5 by NaOH and the sample volume brought to 100 ml with double distilled water (DDW). The GF/C filtrate (1.6 μm pore size), designated as soluble fraction, was divided into two parts. One was immediately used for ASV analysis to determine the concentrations of the free or labile form of the studied cations (SF). The other part was lyophylized and the dry powder digested and treated as described for the particulate fraction. This digested sample was taken as the measure of the total amount of the metals studied in the soluble fraction (free and bound). Appropriate blanks were run for all chemicals used and only the corrected values are presented.

Metal analysis

Differential Pulse Anodic Stripping Voltammetry (DPASV) was used to determine concentration of Zn^{2+},

Cd^{2+}, Pb^{2+} and Cu^{2+} in samples using a hanging mercury drop electrode (HMDE), type E 410 (Metrohm, Switzerland). A calomel electrode fillled with KCl (saturated) served as a reference electrode and a coiled platinum wire was used as an auxiliary electrode. Measurements were made with Model EI224 polarographic analyzer developed and built at the Department of Electrical and Computer Engineering, BGU (Lazer and Ben-Yaakov, 1980). The analysis consists of the following steps: (a) plating the working electrode (WE) at -1400 mV for 120 s in stirred solutions flushed with CO_2. (b) Rest period, unstirred and unflushed, for 30 s at the deposition potential. (c) Scanning from -1400 mV to $+300$ mV (E pulse = 50 mV, E step = 10 mV, T = 640 ms) and recording the current using an x–y plotter. (d) Stripping the WE for 120 s at $+300$ mV in stirred and flushed solution and started again from step (a)–(c) were controlled by a microprocessor, ensuring precise control and reproducibility of critical timing. A fresh mercury drop was used for each plating/stripping cycle. Calibration was done by standard additions of increasing concentrations of the cations to each sample.
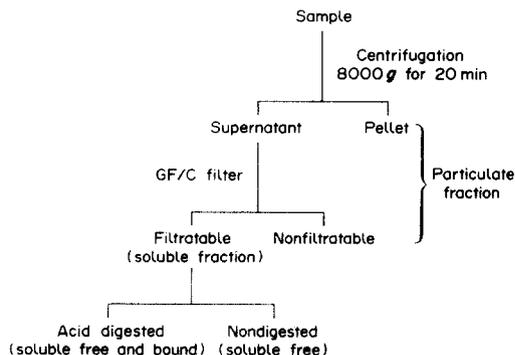

Fig. 2. Flow scheme describing the samples fractionation procedure.

Algal growth conditions

A unicellular green algae, *Chlorella* sp., was isolated from the Beer Sheva stabilization ponds and grown axenically on BG-11 (void of EDTA) (ASM, 1981), at volumes of 100 ml in 250 ml Erlenmayer flasks placed on a rotary shaker at 27°C and illuminated by continuous light of $120 \mu E m^{-2} s^{-1}$ at the surface of the flasks. This growth medium contains 0.222 mg ml^{-1} $ZnSO_4 \cdot 7H_2O$ and 0.079 mg ml^{-1} $CuSO_4 \cdot 5H_2O$. Algal cultures at the end of logarithmic phase of growth were used throughout all experiments unless otherwise stated in the text. *Chlorella* sp. growth was monitored both as absorbance at 540 nm and by cell counts (in a Neubauer Haemocytometer). All reagents were of analytical grade and the solutions were prepared in glass double distilled water.

Proteolysis

Samples were incubated for 2 h at room temperature with 1 mg ml^{-1} protease, type XIV (from *Streptomyces griseus*, Sigma, U.S.A.).

Metal chelating (complexing) capacity

Metal complexing capacity of tested solutions (Shuman and Martin-Goldberg, 1984; Watson, 1968) was determined by standard additions of the cation in question and monitoring the change in the ASV calibration curve. The appearance of a break in the calibration curve of peak current vs the concentration of the added standard was taken as an indication of the presence of a chelating agent which became saturated with the complexed metal at the break point. The total amount of the cation required to reach the break point, therefore, represents the chelating capacity of the original solution.

Water quality analysis

Ammonia, BOD, COD, TSS (Total Suspended Solids) and VSS (Volatile Suspended Solids) were determined according to *Standard Methods* (APHA, 1976). Chlorophyll *a* concentration and oxygen concentration were determined as described elsewhere (Abeliovich and Azov, 1976).

RESULTS AND DISCUSSION

Operational parameters of the Beer Sheva treatment plant are presented in Table 1. The poor performance of the ponds as reflected in terms of BOD and SS is apparently due to overloading of the treatment plant. (For more details see Abeliovich, 1982, 1983.) The total concentrations of the heavy metals as determined by the ASV, after digestion are presented in Table 2. These data are based on a monthly sampling of water at the outlet of the various stages of the plant, during 1984/5. All analyses were done in duplicate. Although absolute values are low, they are significantly higher that those found in the local tap water. The values of total dissolved metal concentrations found here are similar to those previously reported for the raw sewage and effluents of the Ein Karem (Jerusalem) plant (Peleg and Brenner, 1975) which, like the Beer Sheva plant, also handles domestic sewage. The distribution of the metals between particulate and filtered fractions (Table 3) seems to indicate that most of the cations are found in the particulate fraction. During the treatment process, following the decrease in BOD there is a gradual increase in the concentration of Zn, Cu and Pb in the soluble fraction. However, following the decrease in BOD during the treatment process

Table 1. Comparison of water quality, raw wastewater, aerobic/facultative pond effluent, and reservoir. Figures are averages for the whole observation period

	BOD total	BOD filtered	Ammonia concentration (mg l^{-1})	TSS (mg l^{-1})	VSS (mg l^{-1})	Chlorophyll 0 concentration at noon (mg l^{-1})	(mg l^{-1})
Untreated municipal wastewater	220 ± 71	110 ± 30	38 ± 13	215 ± 76	141 ± 5	—	—
Aerobic/ facultative pond effluent	180 ± 37	105 ± 35	47 ± 18	127 ± 35	82 ± 21	0.9 ± 0.5	0.6 ± 0.2
Reservoir water	107 ± 31	47 ± 19	25 ± 51	147 ± 51	101 ± 30	1.2 ± 0.3	7.8 ± 5.2

Table 2. Total amounts of cations in the various stages of the wastewater treatment

	Concentration ($\mu g l^{-1}$)							
	Zn^{2+}		Cd^{2+}		Pb^{2+}		Cu^{2+}	
	$\mu g l^{-1}$	% removed	$\mu g l^{-1}$	% removed	$\mu g l^{-1}$	% removed	$\mu g l^{-1}$	% removed
Raw sewage	224.2 (57.2)	0	2.0 (0.75)	0	53.5 (33.3)	0	34.3 (18.8)	0
Settling pond	171.6 (41.0)	24	1.9 (1.5)	5	43.0 (33.3)	20	59.7 (56.4)	
Anaerobic pond	175.4 (16.0)	22	4.3 (3.3)		38.6 (5.9)	28	29.1 (26.4)	15
Aerobic/facultative pond	203.7 (62.4)	9	2.98 (2.2)		25.3 (2.3)	53	24.1 (16.1)	30
Reservoir	130.0 (44.8)	42	1.1 (0.9)	45	31.7 (5.9)	41	24.2 (15.4)	30
Local tap water	92.2 (23.8)		0.3 (5)		1.3 (1.7)		6.6 (2.9)	

Values in parentheses represent standard deviation.

Table 3. Cations distribution between particulate and filtered fractions during wastewater treatment

	Zn^{2+}		Cd^{2+}		Pb^{2+}		Cu^{2+}	
				Percent of total				
	Part.	Filt.	Part.	Filt.	Part.	Filt.	Part.	Filt.
Raw sewage	91	9	88	12	85	15	75	25
Settling pond	83	17	60	40	48	52	88	12
Anaerobic pond	80	20	62	38	73	27	74	26
Aerpboc/facultative P	84	16	100	0	67	33	73	27
Reservoir	70	30	18	82	75	25	59	41

Table 4. Concentration of free cations at the various stages of the wastewater treatment plants, in filtrate, as determined by ASV

	Concentration ($\mu g\, l^{-1}$)						
	Zn^{2+}		Cd^{2+}		Pb^{2+}		Cu^{2+}
	Average	Range	Average	Range	Average	Range	
Raw sewage	11.2	(< 0.1–40.3)	1.5	(< 0.1–10.0)	3.0	(< 0.1–10.6)	< 0.1
Settling pond	8.8	(< 0.1–40.0)	0.5	(< 0.1–4.0)	3.3	(< 0.1–25.2)	< 0.1
Anaerobic pond	5.5	(< 0.1–11.5)	0.7	(< 0.1–4.0)	5.2	(< 0.1–5.5)	< 0.1
Anaerobic/facultative P	3.7	(< 0.1–10.2)	0.9	(< 0.1–2.8)	3.5	(< 0.1–20.9)	< 0.1
Reservoir	8.1	(< 0.1–30.0)	0.9	(< 0.1–20.0)	6.4	(< 0.1–20.0)	< 0.1

Data based on 9 samples.
Values in parentheses represent minimum and maximum values.

(Table 1), there is a gradual increase in the concentration of Zn, Cu and Pb in the soluble fraction. The inconsistent picture for Cd is apparently due to the fact that the total concentration of this metal was found to be very close to the limits of detection.

The concentrations of the dissolved free cations (Table 4) are about an order of magnitude lower than their total concentrations. It appears, however, that the fractions of free Cu^{2+} and Pb^{2+} are relatively smaller than those of Zn^{2+}, presumably reflecting a higher affinity to these metals of the chelating compound(s) present in the water. No definite conclusion can be drawn regarding Cd^{2+} for the already mentioned reason.

The concentrations of total and free heavy metals found in this study are significantly lower than those reported for the Barrowford (England) water treatment plant (Laxen and Harrison, 1981) which apparently serves a highly industrial area. For example, the concentration of Cu in the raw sewage was reported there to be $80\,\mu g\, l^{-1}$, as compared to $34\,\mu g\, l^{-1}$ which we found in Beer Sheva. The difference is especially large for Cd for which the concentration ratio is about 100 fold: $280\,\mu g\, l^{-1}$ at Barrowford as compared to $2.2\,\mu g\, l^{-1}$ in the Beer Sheva raw sewage. In spite of the large differences in concentrations, the percentage of metal removed throughout treatment is about the same, 50% in both cases. Brown et al. (1973) in their study, monitoring six municipal sewage treatment plants in the U.S., also reported 50% removal for Zn, Pb and Cu, and only 16% for Cd. Apparently the great fluctuations in the environmental conditions prevailing in this biotope did not affect metal distribution in a way which is significantly different from other wastewater treatment facilities. Whatever differences that do exist are overruled by other dominating factors which are common to them all with respect to metal speciation.

Most of the dissolved metals are found in a bound form. For example, in an ASV titration curve of Zn^{2+} in the reservoir water filtrate (Fig. 3A) the fraction of free Zn^{2+} is only about 10% of the total concentration of this metal. When compared to the curve of the digested sample (Fig. 3B). It has been reported (Buffle, 1934; Baccini, 1984) that secretion of polysaccharides, peptides, organic acids, etc., mainly of bacterial origin, are potential chelators for heavy metals.

Laboratory studies on the metal complexing mechanism of the wastewater treatment ponds were performed with axenic cultures of a Chlorella sp. isolated from the same oxidation ponds where it constitutes approx. 80–90% of the total biomass (Abeliovich and Weisman, 1978). We chose to study Zn^{2+} complexing since this metal showed the largest increase in the dissolved fraction during treatment. ASV titration of
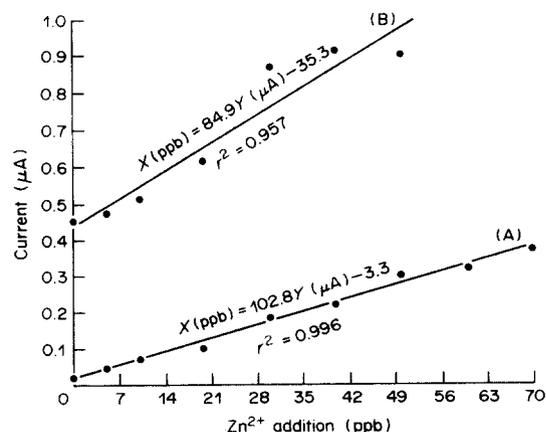

Fig. 3. ASV titration curves of current peak height vs Zn^{2+} concentration. (A) Untreated filtrate of reservoir water, (B) same filtrate after acid digestion.
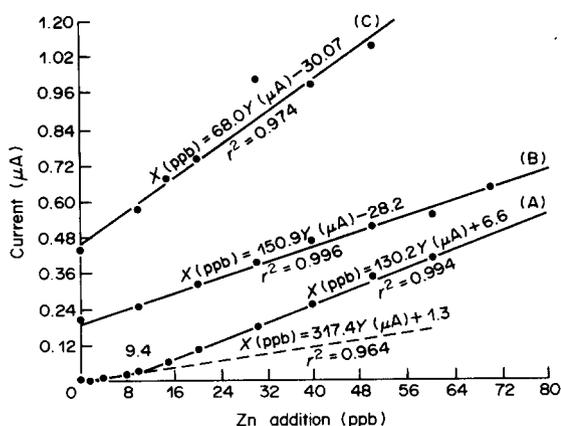
Fig. 4. ASV titration curves of spent growth medium. (A) Untreated control, (B) after proteolysis, (C) after acid digestion.

the spent algal growth medium (Fig. 4A) revealed that Zn is removed from the solution by an algal secretion. The break in the titration curve (Fig. 4A) indicates a surplus complexing capacity of approx. $9.5\,\mu g\,l^{-1}$ (by least square fitting) beyond the concentration of the already bound metal. It was further found that the total Zn^{2+} left in the spent medium (as measured by ASV after acid digestion) was approx. $30\,\mu g\,l^{-1}$ (Fig. 4C). This implies that the equivalent concentration of the chelating capacity for Zn^{+2} was approx. $40\,\mu g\,l^{-1}$. Considering the fact that the Zn concentation in the fresh medium was approx. $60\,\mu g\,l^{-1}$ (Fig. 5A) while that of the spent medium was $30\,\mu g\,l^{-1}$, about $30\,\mu g\,l^{-1}$ has shifted into the particulate fraction, probably into algal cell material. The distribution ratio between the particulate and dissolved cations fractions found in the reservoir was 7:3 (Table 3), which is consistent with our laboratory experiments, even though the total particulate material in the reservoir is higher in the artificial growth medium.
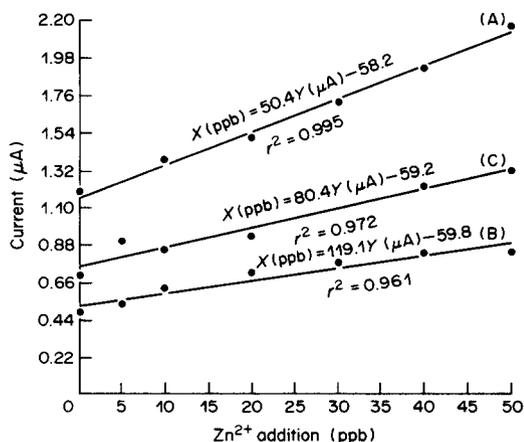

Fig. 5. ASV titration curves of current peak height vs Zn^{2+} concentration fresh medium. (A) Untreated control, (B) after proteolysis, (C) after acid digestion.

The results of the proteolysis experiments on the chelating capacity (Figs 4B, 5B) suggest that the algal secretion responsible for Zn^{2+} complexing is of proteinaceous nature, as the effect of applying protease VIX is very similar to that of total digestion of the same sample of spent growth medium (Fig. 4C). The enzyme by itself does not affect the ASV analysis of the fresh medium except for a slope change (Fig. 5B), as do other proteins such as bovine serum albumin. Similarly Tuschall and Brezonik (1980) reported that a proteinaceous compound isolated from lake water is capable of complexing copper.

The present study suggests that only a small fraction of the dissolved heavy metals is in a labile free form, as determined by ASV. At these low metals concentrations the biota present in the wastewater is not affected. However the fact that the total dissolved and particulate metal forms are transferred to the soil irrigated by these effluents deserves attention although the concentrations of the heavy metals in the present case are relatively low, thanks to the absence of metal-rich influent. Our results also indicate that the effectiveness of metal removed by biological treatment when metal concentration is very low is similar to the effectiveness reported by others (Brown et al., 1973) for high metal concentration effluent, and thus the low efficiency in metal removal observed is not simply a result of the poor performance of the Beer Sheva wastewater treatment plant.

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