Available online at www.sciencedirect.com
ScienceDirect
Journal of Hazardous Materials 158 (2008) 196–201
Journal of Hazardous Materials
www.elsevier.com/locate/jhazmat

Improved derivatisation methods for the determination of free cyanide and cyanate in mine effluent

Caliphs M. Zvinowanda [a,*], Jonathan O. Okonkwo [a], Rogers C. Gurira [b]

[a] *Department of Environmental, Water & Earth Sciences, Faculty of Science, Tshwane University of Technology, Private Bag X680, Arcadia, Pretoria 0001, South Africa*

[b] *Department of Chemistry, Faculty of Science, University of Zimbabwe, Private Bag MP167, Mount Pleasant, Harare, Zimbabwe*

Received 13 November 2007; received in revised form 18 January 2008; accepted 18 January 2008
Available online 26 January 2008

Abstract

Generally, the level of cyanide in waste effluents is too high to be discharged into the environment. Consequently, treatment regimes are necessary in order to protect the environment. However, the cost of most of the treatment methods is expensive and not sensitive enough and, therefore, cannot always be justified. In this research, cyanide speciation products, free cyanide (CN^-) and cyanate (CNO^-) were determined by highly sensitive derivatisation methods followed by spectrometric analysis. Spectral scans were carried out for pure and environmental samples derivatives in order to evaluate the possibility of interfering species. For CN^- a linear range from 0.01 to 80.0 mg/L was determined. In the case of CNO^-, the linear range was between 0.02 and 80.0 mg/L. The detection limits were 0.05 and 0.20 mg/L for CN^- and CNO^-, respectively. These values are in good agreement with those reported in literature. The concentration ranges of the speciation products in environmental samples were 0.70–52.0 mg/L and 0.50–76.0 mg/L for CN^- and CNO^-, respectively. These values were well above their acute toxicity levels. Increase in cyanate levels in the effluent with time was clearly observed while the concentration of cyanide decreased. This was attributed to the oxidation of CN^- to CNO^-.

Keywords: Derivatisation method; Spectrometry; Cyanide; Cyanate; Mine effluent

1. Introduction

Cyanide is a strong ligand capable of complexing with virtually any heavy metal [1]. The complexation of cyanide with heavy metals in organs can totally inhibit all the biochemical processes which may result in human organ failure and even death. The lethal dose of cyanide to human adult is between 50 and 200 mg [2].

Cyanide waste management at most gold mining dump sites involves the monitoring of levels of cyanide and its remediation is by addition of oxidants and complexing agents such as ferrous sulphate which acts as cyanide sinks. The overall toxicity though synergistic, can also depend on the predominant species with concentration above acute toxicity range [3–9].

Different methods for the determination of cyanide and its species have been reported [8–10]. Ion pair/ion interaction chromatography (IIC) has also been used to determine free cyanide (CN^-) from leached liquor [11–14]. However the method is time consuming in sample pretreatment. For the analysis of free cyanides, Guibauilt and Kramer described the reaction of *p*-nitrobenzaldehyde (I) with cyanide in alkaline solution [10]. The method is widely used as a qualitative test since a highly coloured purple compound is formed in the presence of cyanide.

Cyanate is commonly determined using the Kjeldahl nitrogen method. This method requires over 1 h per analysis and involves boiling of concentrated acids. Prior to the development of ion chromatographic method for the analysis of cyanate, a colorimetric method was used [15,16]. This colorimetric method was shown to be unreliable for samples containing low CNO^- in the presence of high concentrations of metals or ammonia. Fagan and Haddad [17] reported an ion chromatographic method which was suitable for samples with Cl^- levels up to 100-fold higher than the CNO^-. However, rapid column deterioration was reported, thus this technique was considered very expensive for routine CNO^- analysis [18,19].

Cyanate is one such anion which is produced during protein poisoning in the body. This anion has been studied extensively in the field of biochemistry because of its toxicity.

* Corresponding author. Tel.: +27 12 382 6281; fax: +27 12 382 6354.
E-mail address: czvino@gmail.com (C.M. Zvinowanda).

0304-3894/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2008.01.059

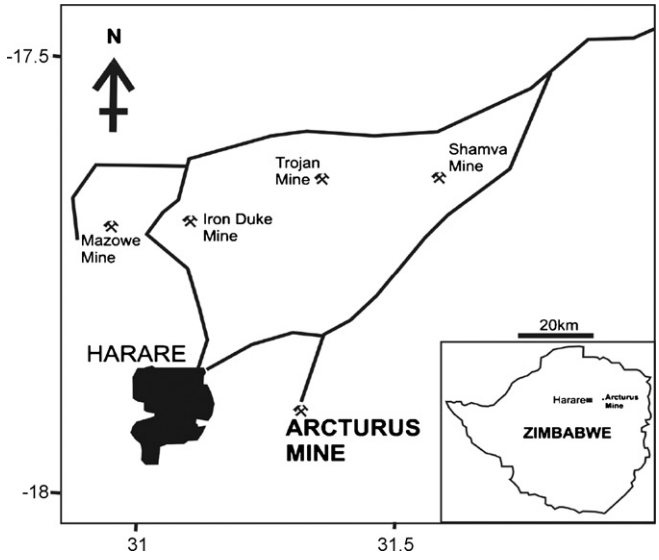


Fig. 1. Map of Zimbabwe (bottom right) and the position of Arcturus Mine (top left).

Cyanate in blood plasma has been determined by the method which involves the derivatisation of cyanate with 2-nitro-5-thiocarbamylbenzoic acid (TNB) [20]. In this paper we report the use of the derivatisation of CNO^- and 2-amino benzoic acid, followed by spectrophotometric method of analysis to determine free cyanate (CNO^-) in environmental samples [23].

2. Experimental

2.1. Location of study area

The location of the study area is shown in Fig. 1, marked as Arcturus Mine.

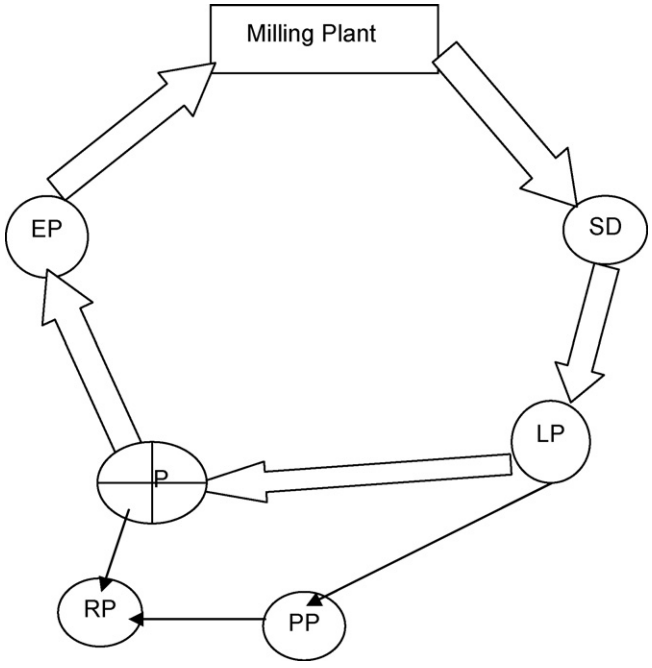


Fig. 2. Flow and seepage of effluent in reservoir ponds. SD: Slime Dam; LP: Lined Pond; PP: Panic Pond; RP: Recollection Point; EP: Evaporation Pond; P: Pump.

Arcturus Mine is located about 40 km northeast of Harare at latitude 17°46′60″S and 31°19′0″E. The mine performs both opencast and underground mining of gold ore and the gold is extracted by the cyanidation process. The effluent from the mine is technically recycled using a system of ponds for storage before pumping back most of the clarified effluent. The following is a general layout of the pond system in which the sampling was carried out over a period of 6 months (Fig. 2).

2.2. Sample collection

Effluent samples were collected randomly in triplicates from the five effluent ponds. The samples were collected in 2.0 L polythene bottles which had been initially thoroughly rinsed with dilute nitric acid and sealed immediately thereafter. Samples were then taken to the laboratory where they were placed in a refrigerator until ready for treatment and analysis. Sample treatment and analysis were carried out at the earliest time possible after collection in order to minimize sample attenuation. Sampling was carried out over a period of 5 months.

2.3. Reagents and chemicals

All reagents and chemicals used in this research were of analytical grades. *p*-Nitrobenzaldehyde (BDH) and *o*-dinitrobenzene (BDH) were used for cyanide determination. For cyanate determination, the reagents used were potassium cyanate (Saarchem, SA), 2-aminobenzoic acid (ABA, SA), 32% HCl (Saarchem, SA) and glacial acetic acid, CH_3CO_2H (Saarchem, SA).

2.4. Sample treatment

2.4.1. Free cyanide

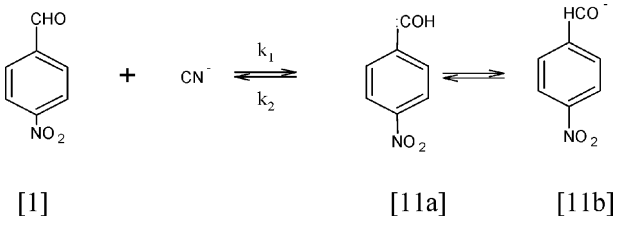
Fifty millilitres (50.0 mL) of each sample was taken for pretreatment before analysis. The pretreatment involved filtration of the sample using 0.45 μm Whitman filter paper to remove particulate matter followed by measurement of pH and adjustment where necessary to pH range 8–9 using 0.10 M NaOH solution or 0.10 M HNO_3 depending on the alkalinity or acidity of the sample, respectively.

2.4.2. Cyanate

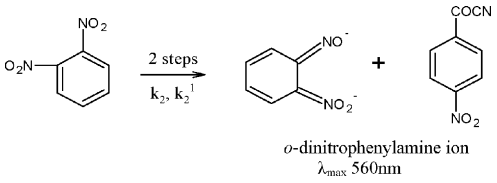
Sample filtration was as described earlier in Section 2.2. Samples with pH above or lower than 7 were adjusted by adding few drops of either 0.10 M HNO_3 or NaOH solutions to pH 7 in order to increase the presence of NH_4^+ in the NH_3/NH_4^+ equilibrium.

2.4.2.1. Derivatisation of free cyanide. Free cyanide was derived as shown in Eq. (1). The derivatisation method was based on the formation of *o*-nitrophenylhydroxylamine anion, with characteristic blue colour with λ_{max} at 560 nm [21,22]. The method involves forming a mixture consisting of 1.0 mL of 0.01 M *o*-dinitrobenzene and *p*-nitrobenzalaldehyde, 0.10 mL of 0.50 M $NaOH_{(aq)}$ and 0.10 mL of 0.10 M of cyanide added to the mixture to initiate the reaction. The mixture was thoroughly stirred and allowed to stand for varied 15–20 min. However,

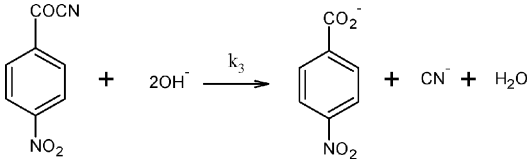
about 15 min was found to be the best reaction time for the mixture. The absorbance of *o*-dinitrophenylamine was continuously increasing with time and this was recorded as a function of time. The same procedure was repeated for the standards. Plots of absorbance against time were constructed and the initial rate for each cyanide sample was determined [24–26]. The concentrations of cyanide in each sample were read from the calibration curve of initial rates of standards versus concentration of standards. A mixture consisting 1.0 mL each of 0.01 M *o*-dinitrobenzene and *p*-nitrobenzalaldehyde and 0.10 mL of 0.50 M $NaOH_{(aq)}$ was placed in a cuvette and its absorbance was adjusted to zero as measurement blank.



[1] [11a] [11b] (1)



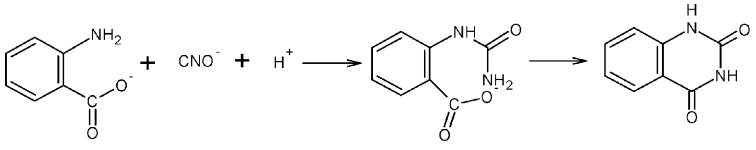
o-dinitrophenylamine ion
λ_{max} 560nm (2)



(3)

2.5. Derivatisation of cyanate

Cyanate was derivatised as shown in Eq. (4). The assay of cyanate in this analysis was based on a specific method where by cyanate reacted with 2-aminobenzoic acid, in the presence of HCl and glacial acetic acid, CH_3CO_2H to form quinazolinedione [23].



2-ABA 2,4-(1*H*, 3*H*)-quinazolinedione
λ_{max}= 310nm (4)

The product 2,4-(1*H*, 3*H*)-quinazolinedione formed, absorbed strongly in the ultraviolet region at 310 nm. The reaction was found to be pH-dependent and as a result the reaction was performed at pH range 1–6 for optimization. pH of <2 was found to be appropriate at room temperature(25 °C). The absorbance of standards and samples were also measured at 310 nm after reaction [23,31,33,34].

2.6. Instrumentation

A PerkinElmer Lambda-2-UV–vis Spectrometer was used in the quantitative analysis of all the two cyanide species investigated. The instrument was initially adjusted to zero by the use of appropriate blanks of each sample. Glass cuvettes were used for all other samples and their standards since these absorb in the visible region. For the cyanate derivative, a quartz cuvette was used since the analytical wavelength was 310 nm in the UV region. Cyanide analysis was measured at 560 nm where the intermediate derivative had its absorption maxima.

2.7. Quality assurance

All reagents were prepared from analytical grade materials according to specific procedures using appropriate solvents, deionised and distilled water depending on the protocol [27–29]. The formation of derivatives was qualitatively confirmed by obtaining their spectra through scanning from 200 to 750 nm in order to identify their absorption maxima. Blanks for each analysis were prepared by mixing all the reagents and solvents in appropriate proportions leaving out the analyte which was either the sample or the standard in each case. Most standards were freshly prepared except in cases where the protocols indicated otherwise. Recovery tests were carried out by spiking pre-extracted environmental samples with known amounts of standards of cyanide and cyanate, respectively to test the reliability of the methods used.

2.8. Statistics

One-way analysis of variance (ANOVA) was used to test whether there was significant variation of cyanide species between sampling months. Mean concentrations of the samples were calculated from various months using Microsoft EXCEL.

3. Results and discussion

The method for cyanide determination had a linear range from 0.01 to 80.0 mg/L and 0.02 to 80.0 mg/L for CN^- and CNO^-, respectively. Their regression coefficients ranged from 0.987 to 0.998. The detection limits were 0.05 and 0.20 mg/L for CN^- and CNO^-, respectively based on a signal to noise ratio of 4 [32,35].

Recovery tests using the derivatisation method were carried out with different samples, and the test for each sample was conducted in triplicate. As shown in Table 1, the % recoveries

Table 1
Results of recovery test of spiked pre-extracted real samples

Sample	Amount added (mg/L ± R.S.D.)		Amount recovered (mg/L ± R.S.D.)		Recovery (% ± R.S.D.)	
	CN^-	CNO^-	CN^-	CNO^-	CN^-	CNO^-
SD	4.00 ± 0.12	5.00 ± 2.10	3.60 ± 1.80	4.25 ± 1.77	90.0 ± 1.2	85.2 ± 0.7
LP	1.50 ± 0.10	2.50 ± 0.86	1.44 ± 1.60	2.33 ± 1.42	96.4 ± 0.8	93.1 ± 1.3
PP	0.20 ± 0.20	2.00 ± 0.52	0.18 ± 0.65	1.88 ± 0.84	92.3 ± 0.5	94.0 ± 0.4
RP	0.40 ± 0.15	0.50 ± 0.44	0.35 ± 0.40	0.45 ± 0.25	88.1 ± 2.1	90.3 ± 0.6
EP	1.00 ± 1.70	3.00 ± 0.23	0.90 ± 0.35	2.76 ± 0.44	90.3 ± 0.9	92.2 ± 0.5

SD = Slime Dam; LP = Lined Pond; PP = Panic Pond; RP = Recollection Point; EP = Evaporation Pond.

ranged from 90 to 96% for cyanide and 85–93% for cyanate. The high percentage recoveries validated the derivatisation method used in the present study.

A comparison of the results obtained by derivatisation, ion-chromatography and Kjeldahl method for cyanate in Slime Dams is shown in Table 2. From Table 2, the values obtained using DM ranged from 0.15 to 7.6. This is closer to the range for IC. Considering the high demand for sample preparation, for IC, DM which is selective and requires minimal sample treatment hence, offers a good alternative for cyanate analysis. However, the values obtained using KJ as reported by Black and Schulz [32] ranges from 5 to 13. These values are significantly higher than the derivatisation range obtained in this study. The higher range exhibited by KJ may be due to the conversion of SCN^-, CN^-, and NH_3 according to the following equations:

$$CN^-_{(aq)} + 2H_2O_{(l)} \rightarrow NH_{3(aq)} + HCOO^-_{(aq)} \quad (5)$$

$$SCN^-_{(aq)} + 2H_2O_{(l)} + (5/2)O_2 \rightarrow NH_{3(aq)} + HCO_3{}^-_{(aq)} + SO_4{}^{2-}_{(aq)} \quad (6)$$

$$2CNO^-_{(aq)} + 2H^+_{(aq)} + 4H_2O_{(l)} \rightarrow 2NH_4{}^+_{(aq)} + 2HCO_3{}^-_{(aq)} \quad (7)$$

These reactions take place in the presence of strong acid which is one of the conditions in KJ reaction. This then suggests that KJ is non-selective.

Figs. 3–5 show the graphs of three batches of samples which were analysed for cyanide and cyanate between April and August 2002, respectively. From Fig. 3 there was a clear pattern in the variation of cyanide and cyanate in the five batches of samples analysed. It can be observed that cyanide and cyanate concentrations decreased from the Slime Dam (SD), Lined Pond (LP), Panic Pond (PP) and Recollection Pond (RP) for cyanate, respectively. Generally the levels of cyanide were generally lower than those of cyanate in the respective tailing ponds, except the Recollection Pond. The Slime Dam which is the receiving dam of fresh tailing waste which is made up of slurries, showed the highest levels of cyanide and cyanate for all the five ponds sampled. This was followed by the Lined Pond and the Panic Pond. Thereafter, there was a gradual increase up to the Evaporation Pond (EP). The same pattern was also repeated for cyanate. The decrease in the cyanide levels observed up to the Panic Pond could be ascribed to physiochemical processes whereby cyanide attenuated possibly into NH_3, CNO^- and SCN^- [30]. This could also explain why the values of CNO^- were significantly higher than those of CN^-. Also, some of the cyanide could have been lost through volatilization into the atmosphere. The effluent from SD is channeled to the LP through underground piping after the separation of the slurries into soil and wastewater. The reduction

Table 2
Comparison of the DM with IC and KJ for cyanate determination in Slime Dams in mg/L ± R.S.D.

Sample	$[CNO^-]$ DM	$[CNO^-]$ IC	$[CNO^-]$ KJ
1	0.15 ± 0.60	2.69 ± 0.40	5.00 ± 1.50
2	0.12 ± 0.20	3.83 ± 0.50	5.59 ± 2.00
3	3.50 ± 0.80	5.66 ± 0.30	8.09 ± 0.90
4	3.50 ± 1.10	7.00 ± 0.80	12.6 ± 2.20
5	7.60 ± 0.90	6.12 ± 0.70	10.0 ± 1.50

DM = derivatisation method; IC = ion chromatography; KJ = Kjeldahl; R.S.D. = relative standard deviation.

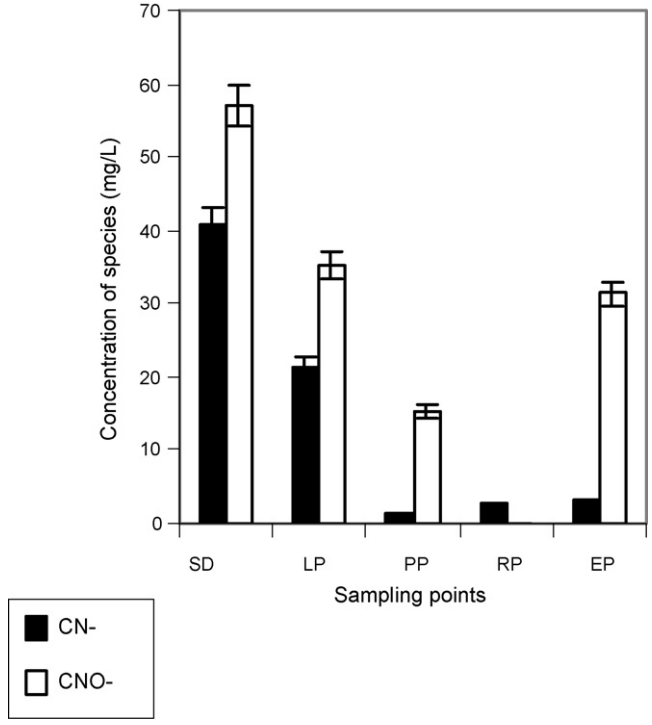


Fig. 3. Variation of CN^- and CNO^- in the Slime Dams in April 2002.

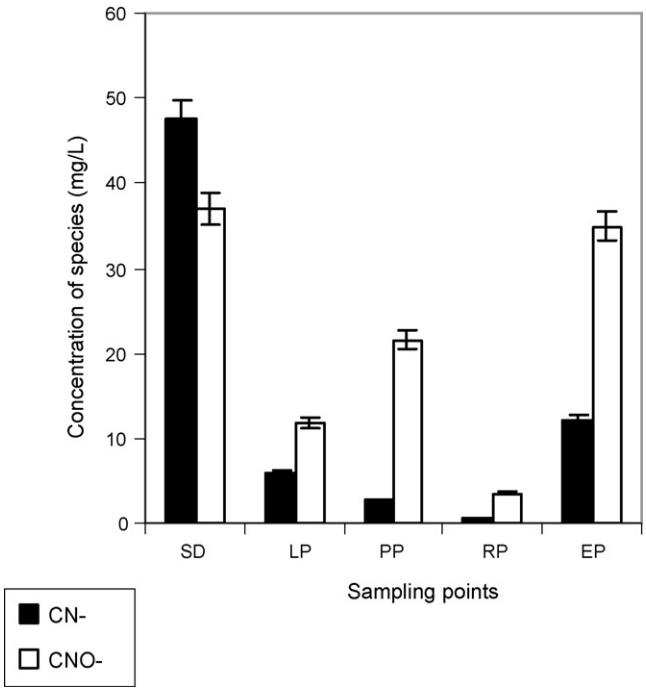


Fig. 4. Variation of CN^- and CNO^- in the Slime Dams in June 2002.

of CNO^- to species like CO_3^{2-} and NH_4^+ could be less rapid in this alkaline range.

In Fig. 4 the pattern as observed in Fig. 3 with respect to CN^- was repeated. However, the decrease in CN^- extended to RP instead of at PP as observed in Fig. 3. It can be observed that the level of CN^- increased at EP. The level of cyanide in the SD was higher in Fig. 4 and CNO^- compared to the observation made in Fig. 3. The high level of CN^- could be associated to the cool temperatures during this period of the year. Consequently, volatilization was very much reduced and hence the loss of CN^- was minimal.

The results of August analysis are shown in Fig. 5. From Fig. 5 the levels of CN^- were significantly lower than those of CNO^- except for RP. This is not surprising since the month of August is fairly warm and windy. The volatilization of CN^- is therefore expected to be fairly high, hence the observed low values. There was again a general decrease of CNO^- from SD to RP and an increase at EP as observed in Figs. 3 and 4. However, with CN^-, the SD exhibited the lowest level and LP the highest level. The levels of CN^- at RP and EP can be considered to be more or less the same. From Figs. 3–5 it can be observed that RP exhibited the lowest levels of CN^- and CNO^-. This can be attributed to the fact that most of the wastewater found at this point comes through seepage. The mobility of the species into the RP may have been restricted through this mechanism. Also from Figs. 3–5, the increase in CNO^- in EP could be due to favourable pH and longer residence time in this reservoir.

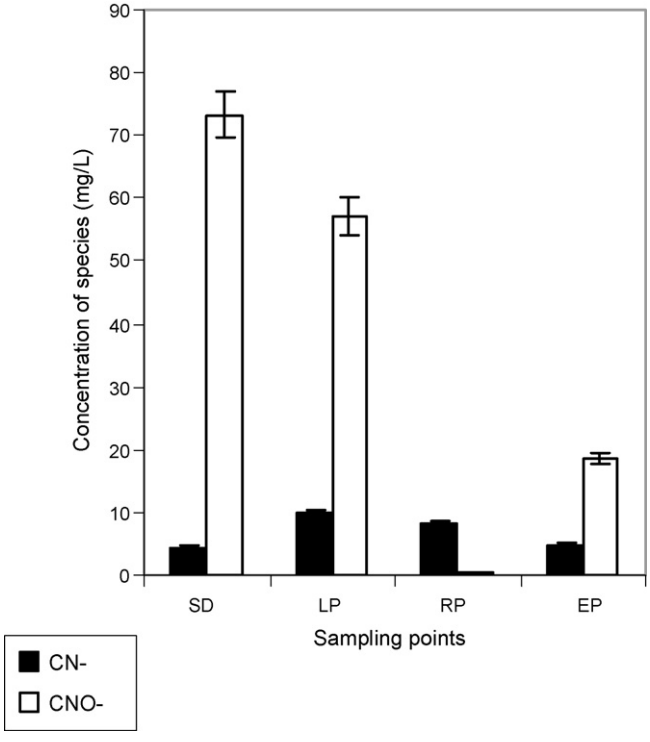


Fig. 5. Variation of CN^- and CNO^- in the Slime Dams in August 2002.

4. Conclusion

The derivatisation methods used in the present study for cyanide and cyanate analyses had detection limits of 0.05 and 0.20 mg/L for cyanide and cyanate, respectively. These values are in good agreement with those reported in the literature. Validation of the method using spiking methods showed that the derivatisation method could be applied to environmental samples with reasonable reliability.

Acknowledgement

The authors are indebted to the Department of Chemistry, Faculty of Science, University of Zimbabwe, for financial support.

References

[1] American Public Health Association, Standard Methods for the Examination of Water and Wastewater, 19th ed., American Public Health Association, Washington, DC, 1995.
[2] R.P. Brown, M.R. Lanno, V.D. Heuvel, D.G. Dixon, HPLC determination of plasma thiocyanate concentrations in fish blood: application to laboratory pharmacokinetic and field-monitoring studies, Ecotoxicol. Environ. Safe. 30 (1995) 302–308.
[3] Colorado Department of Natural Resources, Guidelines for the Characterisation, Monitoring, Reclamation and Closure of Cyanide Leaching Projects—"Cyanide Guidelines for Colorado", Colo. Div. of Mines and Geology, Denver, CO, November 1999.
[4] D.G. Dixon, G. Leduc, Chronic cyanide poisoning of rainbow trout and its effects on growth, respiration and liver histopathology, Arch. Environ. Contam. Toxicol. 10 (1981) 117–131.
[5] D.G. Dixon, J.B. Sprague, Acclimation-induced changes in toxicity of arsenic and cyanide to rainbow trout *Salmo gairdneri* Richardson, J. Fish Biol. 18 (1981) 579–589.
[6] J.C. Blailar (Ed.), Comprehensive Inorganic Chemistry, Perganon Press, Oxford, 1973.
[7] A. Scattergood, Ammonium cyanate, Inorg. Synth. 2 (1946) 88.

[8] B.J. Moody, Comparative Inorganic Chemistry, Edward Arnold Ltd., London, 1965, p. 283.
[9] Kloepper, Cyanates of alkali metals, US Patent 1,915,425 (June 27, 1933), Chem. Absracts, 27 (1933) 4354.
[10] G.G. Guibauilt, D.N. Kramer, Ultra sensitive specific method for cyanide determination using *p*-nitrobenzaldehyde and *o*-dinitrobenzene, Anal. Chem. 38 (7) (1966) 835.
[11] O. Lška, J. Lehotay, E. Brandšteterová, G. Guiochon, H. Colin, Liquid chromatography of metal complex of N-disubstituted dithiocarbamic acids. IV. Separation of mixtures of Zn(II), Cu(II), Mn(II), Ni(II), Pb(II), Cr(III), Co(II), Cd(II) and Fe(II) diethyldithiocarbamate complexes by high-performance liquid chromatography, J. Chromatogr. 172 (1) (1979) 384–387.
[12] P.R. Haddad, C. Kalambaheti, Advances in ion chromatography: speciation of μl^{-1} levels of metallo-cyanides using ion-interaction chromatography, Anal. Chim. Acta 250 (1991) 21–36.
[13] D.F. Hilton, P.R. Haddad, Determination of metal-cyano complexes by reversed-phase ion-interaction high-performance liquid chromatography and its application to the analysis of precious metals in gold processing solutions, J. Chromatogr. 361 (1986) 141–150.
[14] P. Heizmann, K. Ballschmiter, Chromatography of metal chelates. VII. High-performance liquid chromatography of metal 1,2-diketobisthiobenzhydrazones, metal dialkyldithiocarbamates and metal 1,2-diketobisthiosemicarbazones, J. Chromatogr. 137 (1977) 153–163.
[15] M. Ghaedi, H. Parham, M. Moghadam, K. Mortazavi, M. Soylak, Solid phase extraction and spectrophotometric determination of trace amounts of thiocyanate in real samples, Ann. Chim. 96 (11–13) (2006) 689–696.
[16] A. Liberatori, T. Lanoce, L. Loreti, M. Pettine, Selective determination of organic and inorganic thiocyanates in aqueous solution by IR spectrophotometry, Int. J. Environ. Anal. Chem. 22 (1985) 109–114.
[17] P.A. Fagan, P.R. Haddad, Determination of free cyanide in gold cyanidation process liquors by ion-interaction chromatography with post-column derivatisation, J. Chromatogr. 550 (1991) 559–571.
[18] N.J. Csikai, A.A. Barnard Jr., Determination of total cyanide in thiocyanate containing wastewaters, Anal. Chem. 55 (1983) 1677.
[19] A. Smith, T. Mudder, Chemistry and Treatment of Cyanidation Wastes, Mining Journal Books Ltd., 1991.
[20] V. Balzani, V. Carassiti, Photochemistry of Coordination Compounds, Academic Press, London, 1970, pp. 147–148.
[21] A.K. Holliday, Comprehensive Inorganic Chemistry, Perganon Press, Oxford, 1973.
[22] D.N. Kramer, G.G. Guibauilt, The reduction of 1,2-dinitrobenzene by the cyanohydrin of *p*-nitrobenzaldehyde, J. Org. Chem. 31 (1966) 1103.
[23] M. Guilloton, F. Karst, A spectrophotometric determination of cyanate using reaction with 2-aminobenzoic acid, Anal. Biochem. 149 (1985) 291–295.
[24] Standards Methods for the Examination of Water and Wastewaters, 20th ed., APHA, ANWA, WEF, Washington, DC, 1998.
[25] J.F. Thompson, G.R. Morrison, Determination of organic nitrogen, Anal. Chem. 23 (8) (1951) 1155–1157.
[26] J.A. Dean (Ed.), Lange's Handbook of Chemistry, McGraw-Hill Book Co., New York, 1985.
[27] E.M. Robert, Some observations on the chemistry, toxicity and analysis of mining related waters, Mineral Policy Institute, Golden, Colorado, USA, 2004.
[28] S.N. Wiemeyer, E.F. Hill, J.W. Carpenter, A.J. Krynitsky, Acute oral toxicity as sodium cyanide in birds, J. Wildl. Dis. 22 (4) (1986) 538–546.
[29] C.A. Young, T.S. Jordan, Cyanide remediation: current and past technologies, in: Proceedings of the 10th Annual Conference on Hazardous Waste Research, 1995.
[30] R.C. Gurira, Study of cyanide speciation and its environmental impact on quality of water around gold mine (Research Report 2000).
[31] N.A. Lange, F.E. Sheilbey, Benzolylene urea [2,4(1,3)]-quinazolinedione, Org. Synth. 2 (1962) 79–80.
[32] S.B. Black, R.S. Schulz, Ion chromatographic determination of cyanate in saline gold processing samples, J. Chromatogr. A 855 (1999) 267–272.
[33] P.R. Haddad, Ion chromatographic analysis of cyanate in gold processing samples containing large concentrations of copper(I) and other metallocyanide complexes, J. Chromatogr. A 770 (1997) 175–183.
[34] M.-F. Lin, Ion chromatographic quantification of cyanate in urea solutions: estimation of the efficiency of cyanate scavengers for use in recombinant protein manufacturing, J. Chromatogr. B 803 (2004) 353–362.
[35] M. Noroozifar, M. Khorasani-Motlagh, R. Zare-Dorabei, Application of AgX (X = SO_3^{2-}, $Cr_2O_7^{2-}$, $C_2O_4^{2-}$ and CO_3^{2-}) solid-phase reagents for indirect determination of cyanide in industrial effluent using FIA-FAAS system, Talanta 72 (2007) 1773–1778.