Wastewaters from ethylenediamine production and assessment of their toxicity by bioindicator testing

N A Bykovsky, E A Kantor, L N Puchkova, P A Rahman and N N Fanakova
Ufa State Petroleum Technological University, 1, Kosmonavtov Street, Ufa, 450062, Bashkortostan, Russia
E-mail: nbikovsky@list.ru

Abstract. This article discusses the occurrence of toxic wastewater from the production of ethylenediamine by aminating 1,2-dichloroethane with ammonia. It is shown that wastewater from ethylenediamine production is formed at the stages of evaporation of ethylenediamine dihydrochloride and rectification of a mixture of amines obtained as a result of evaporation. In the first case, the wastewater contains a saturated NaCl solution with a content of 1÷2% of polyethylene polyamines, and in the second case, the wastewater is a solution containing about 1% of ethylenediamine and about 2% of ammonia. To study the toxicity of these wastewaters, watercress (Lepidium sativum) varieties Zabava and Krupnolistovoy were used. The assessment of toxic properties of wastewater was carried out according to such indicators as seed germination, the average length of seedlings and the dry weight of seedlings. It is revealed that the studied wastewaters have an acute toxic effect on the watercress of both varieties. It is shown that the regression equations obtained for the dry weight of seedlings and seed germination, in contrast to the length of the seedlings, do not adequately describe the experimental results and cannot be used to determine the safe dilution ratio. The safe dilution ratio, calculated using the average length of seedlings, ranges from 489.1 to 892.9 for various runoffs and watercress varieties. It is shown that the most toxic is the runoff containing a saturated NaCl solution with a content of 1÷2% polyethylene polyamines. Zabava is the most sensitive to the degree of effluent toxicity.

1. Introduction
Due to its unique properties, ethylenediamine (EDA) has found its use in various fields. EDA using is required to obtain some antioxidant additives to motor oils, latex stabilizers, fungicides, plasticizers, etc. [1–3]. EDA is synthesized by the reaction of 1,2-dichloroethane and ammonia [4-7]. As a result of the synthesis, ethylene diamine dihydrochloride is formed:

\[ \text{C}_2\text{H}_4\text{Cl}_2 + 2\text{NH}_3 \rightarrow \text{C}_2\text{H}_4(\text{NH}_2)_2 \cdot 2\text{HCl}. \]

As it is shown in [6, 7], the reaction of dichloroethane and ammonia proceeds in 20÷70% aqueous solution. The following parameters must be observed: the process temperature is about 1800 °C, the pressure varies from 0.8 to 7 MPa, the molar ratio of dichloroethane to ammonia is 1:2÷6.4.

To isolate ethylenediamine, the dihydrochloride is destroyed with sodium hydroxide:

\[ \text{C}_2\text{H}_4(\text{NH}_2)_2 \cdot 2\text{HCl} + 2\text{NaOH} \rightarrow \text{C}_2\text{H}_4(\text{NH}_2)_2 + 2\text{NaCl} + 2\text{H}_2\text{O}. \]

The result is an aqueous solution, which consists of ethylene diamine, polyethylene polyamines and sodium chloride. Further, this solution is fed to the evaporation and rectification [7]. The evaporation
leads to the formation of gaseous ammonia, amines solution and saturated solution of sodium chloride with a content of 1-2% polyethylene polyamines. Gaseous ammonia is used to obtain an aqueous solution used in the amination process. The amines solution for its separation is rectified. During the rectification an aqueous fraction containing up to 1% ethylene diamine and up to 2% ammonia forms in the upper part of the column.

In the first case, an effluent is formed, which contains a saturated solution of sodium chloride mixed with polyethylene polyamines. In the second case, it is an effluent containing ethylenediamine and ammonia. Production of 1 ton of ethylenediamine is accompanied by the formation of up to 3 m³ of effluent from the evaporation stage and up to 14 m³ of effluent from the rectification stage. The concentration of amines, ammonia and salt in these runoffs is much higher than their maximum allowable concentrations. The purpose of the research is to assess the waste toxicity and the degree of its dilution before discharge.

Toxicity of wastewater was determined by bioindicator testing, using 2 varieties of watercress as a phyto-indicator [8-16]. Experiments were carried out according to the method [17].

The results of studies of the wastewater toxicity, formed in the upper part of the rectification column and containing ethylenediamine and ammonia, as well as wastewater formed during the evaporation stage and containing saturated NaCl solution and polyethylenepolyamines, are shown.

2. Material and research methods
The toxicity analysis of wastewater from ethylene diamine production was carried out with two effluents. The first effluent contained 1% of ethylenediamine and 2% of ammonia. The second effluent contained a saturated solution of NaCl with 2% polyethylene polyamines. The watercress varieties Krupnolistovoy and Zabava were used as the phyto-indicators. Experiments carried out as follows: 30 seeds of the plant were placed on the filter paper, placed in Petri dishes and moistened with 5 ml of the test solution.

The analyzed solution was the undiluted wastewater and the same solution diluted by a factor of 2, 4, 8, 16, 32, 64, 128, 256, 512 and 1024. In the control sample, the filter paper was moistened with distilled water.

Each experiment was conducted in 3-fold repeatability. After eight days, seeding was evaluated, dry weight and length of watercress sprouts were measured [17]. The relative measurement error was determined from the results of each experiment. In this case, the confidence probability was 95%. According to the method [17, 18], a linear regression equation was used to obtain the dependence of the parameters under study on the dilution degree.

To determine the effect of ethylene diamine and polyethylene polyamines on the toxicity of wastewater, experiments were performed with a 2% ammonia solution and with a saturated NaCl solution. The cress Zabava was used as a phyto-indicator.

3. Experimental results and discussion
The effect of the wastewater dilution factor on seed germ is shown in Tables 1 (var. Zabava) and 2 (var. Krupnolistovoy).

Seed germination begins with a 16-fold dilution of wastewater. At the same time, germination ranges from 29.7% to 40.6% at 16-fold dilution of effluent. Further dilution of wastewater leads to an increase in seed germination to a value of 98.9%. However, starting from 32-fold dilution, the dependence of seed germination on the effluent dilution ratio is not observed. This is fair for both the Zabava and the Krupnolistovoy varieties.

The magnitude of the error in determining the germination of seeds of cress of both varieties vary widely from 3.4% to 65.5%. It should be noted that the seed germination values in the case of the control sample both for wastewater containing NH₃ + EDA and for the wastewater containing NaCl + PEP differ from each other by no more than 1.2%. This indicates a fairly good reproduction of the results obtained in identical conditions.
Table 1. Effect of the wastewater dilution factor on the seeds germ of cress var. Zabava.

| Dilution | Effluent, containing NaCl + PEPA | Effluent, containing NH₃ + EDA | Saturated NaCl solution | 2% Ammonia solution |
|----------|---------------------------------|-------------------------------|------------------------|---------------------|
|          | Germinating, %                  | Germinating, %               | Germinating, %         | Germinating, %      |
|          | Error, %                        | Error, %                     | Error, %               | Error, %            |
| 16       | 32.3                            | 19.5                         | 42.7                   | 23.6                |
| 32       | 84.5                            | 11.2                         | 95.4                   | 5.1                 |
| 64       | 88.8                            | 3.5                          | 98.8                   | 4.8                 |
| 128      | 97.9                            | 7.4                          | 96.7                   | 14.9                |
| 256      | 97.6                            | 7.5                          | 94.3                   | 10.3                |
| 512      | 92.4                            | 15.3                         | 95.6                   | 5.2                 |
| 1024     | 98.8                            | 3.4                          | –                      | –                   |
| Control  | 93.5                            | 22.2                         | 94.5                   | 13.5                |

Table 2. Effect of the wastewater dilution factor on the seeds germination of cress var. Krupnolistovoy.

| Dilution | Effluent, containing NaCl + PEPA | Effluent, containing NH₃ + EDA |
|----------|---------------------------------|-------------------------------|
|          | Germinating, %                  | Germinating, %               |
|          | Error, %                        | Error, %                     |
| 16       | 29.7                            | 18.7                         |
| 32       | 89.9                            | 17.9                         |
| 64       | 90.0                            | 6.8                          |
| 128      | 94.5                            | 7.6                          |
| 256      | 93.3                            | 11.4                         |
| 512      | 92.2                            | 7.7                          |
| 1024     | 94.2                            | 25.2                         |
| Control  | 93.6                            | 11.4                         |

To assess the possibility of using the experimental results of seed germination in determining the safe dilution ratio of runoff, the regression equations for the seed germination dependence on the dilution ratio were calculated. In accordance with the methodology [17, 18] the linear regression equation was used to process the experimental results. The correlation coefficients between the experimental results and the equations obtained for its description were calculated. The regression equations and correlation coefficients are shown in table 3.

Table 3. Regression equations and correlation coefficients of seed germination depending on the dilution ratio.

| Effluent | Zabava | Krupnolistovoy |
|----------|--------|---------------|
|          | Regression equation | Correlation | Regression equation | Correlation |
| NH₃ + EDA | Y = 96.63 – 0.003·X | 0.212 | Y = 72.15 + 0.049·X | 0.347 |
| NaCl + PEPA | Y = 90.40 + 0.008·X | 0.465 | Y = 92.71 – 0.005·X | 0.288 |
| Saturated NaCl solution | Y = 49.70 + 0.052·X | 0.431 | – | – |
| 2% ammonia solution | Y = 32.06 + 0.113·X | 0.409 | – | – |

Here, Y – seed germination, %; X is the multiplicity of dilution.

It is known [18] that the critical values of the sample correlation coefficient at 95% confidence probability for 18 experiments (NH₃ + EDA sink) are 0.468, and for 21 experiments (NaCl + PEPA sink) are 0.433. Analysis of the results shows that the correlation coefficients are in the range from 0.212 to 0.465. This indicates that the regression equations of seed germination dependencies for both cress...
varieties on the dilution ratio do not describe the experimental data reliably. Thus, the use of the dependence of the germination of watercress seeds on the dilution ratio cannot reliably determine the safe dilution ratio for both effluents. This is true for a saturated solution of NaCl and for a 2% solution of NH₃.

Tables 4 and 5 show the average length of seedlings of watercress seeds at various wastewater dilution ratios for each parallel experiment.

The minimum length of watercress seedlings is 6.12 mm and is observed when the multiplicity of dilution of the effluent is 16. The maximum length of the seedlings of watercress is 115.61 mm and is observed when the multiplicity of dilution is 512.

There is a clear dependence of the length of seedlings, both on the composition of the wastewater, and on the cress plants. So for the effluent containing a saturated solution of NaCl with 2% PEPA, the average seedling length is less than for a runoff containing 1% ED and 2% NH₃. The difference in the length of the seedlings is 58.7% for Zabava variety and 41.4% for Krupnolistovoy variety.

The error in determining the average length of seedlings for all experiments has approximately the same values and varies from 6.33% to 15.62%. This fact indicates a fairly high reproducibility of this parameter. At the same time, there are no visible deviations in the seedlings length for a saturated solution of NaCl and 2% NH₃ solution.

### Table 4. Dependence of the average length of seedlings of cress seeds of Zabava variety on the dilution ratio.

| Dilution | Effluent, containing NH₃ + EDA | Effluent, containing NaCl + PEPA | 2% Ammonia solution | Saturated NaCl solution |
|----------|-------------------------------|---------------------------------|---------------------|------------------------|
|          | Length, mm  | Error, %  | Length, mm  | Error, %  | Length, mm  | Error, %  | Length, mm  | Error, %  |
| 16       | 29.14       | 10.36     | 30.52       | 12.54     | 25.68       | 11.48     | 8.34         | 10.3      | 10.25       | 5.2       |
| 32       | 38.21       | 9.43      | 34.86       | 9.78      | 41.07       | 13.04     | 25.37        | 11.2      | 18.41       | 4.9       |
| 64       | 77.10       | 13.37     | 69.86       | 9.88      | 80.62       | 11.55     | 81.24        | 9.4       | 83.25       | 9.5       |
| 128      | 73.11       | 10.14     | 80.54       | 7.67      | 85.27       | 8.35      | 85.31        | 7.1       | 107.47      | 8.3       |
| 256      | 84.82       | 10.03     | 85.70       | 8.35      | 83.33       | 11.04     | 90.27        | 5.3       | 115.26      | 7.8       |
| 512      | 85.58       | 10.59     | 97.38       | 9.17      | 100.32      | 9.2       | 146.39       | 10.6      |
| 1024     | –           | –         | 98.80       | 8.08      | –           | –         | 136.83       | 11.8      |
| Control  | 99.13       | 11.27     | 99.32       | 11.34     | 97.46       | 8.6       | 121.27       | 9.2       |
Table 5. Dependence of the average length of cress seedlings of the Krupnolistovoy variety on the dilution ratio.

| Dilution | Effluent, containing NH₃ + EDA | Effluent, containing NaCl + PEPA |
|----------|---------------------------------|----------------------------------|
|          | Length, mm | Error, % | Length, mm | Error, % |
| 16       | 11.12      | 14.91    | 6.12       | 10.25    |
|          | 14.40      | 12.59    | 8.31       | 11.67    |
|          | 14.73      | 15.62    | 9.15       | 9.84     |
| 32       | 41.38      | 11.80    | 24.17      | 14.35    |
|          | 43.63      | 10.52    | 22.54      | 12.56    |
|          | 45.89      | 13.05    | 23.33      | 14.88    |
| 64       | 61.74      | 13.05    | 62.82      | 10.42    |
|          | 60.21      | 6.33     | 73.89      | 13.18    |
|          | 62.94      | 9.31     | 70.96      | 16.15    |
| 128      | 65.13      | 15.01    | 80.03      | 7.67     |
|          | 79.14      | 8.40     | 87.76      | 14.83    |
| 256      | 94.79      | 15.24    | 84.30      | 14.84    |
|          | 89.47      | 16.58    | 96.90      | 11.04    |
|          | 95.03      | 13.47    | 83.93      | 10.06    |
| 512      | 106.59     | 10.39    | 96.11      | 13.23    |
|          | 115.61     | 11.46    | 92.89      | 13.73    |
|          | 108.64     | 15.09    | 97.48      | 11.59    |
| 1024     | –          | –        | 103.62     | 9.87     |
|          | 102.41     | 9.74     | 103.52     | 11.66    |
| Control  | 122.53     | 11.01    | 114.96     | 7.62     |
|          | 124.33     | 9.81     | 106.46     | 8.21     |

Table 6 represents the regression equations and the correlation coefficients of the dependence of the average length of seedlings on the dilution ratio for both effluents.

Table 6. Regression equations and correlation coefficients of the dependence of the average length of seedlings on the dilution ratio.

| Effluent       | Zabava | Krupnolistovoy |
|----------------|--------|----------------|
|                | Regression equation | Correlation | Regression equation | Correlation |
| NH₃ + EDA      | $Y = 49.78 + 0.093 \cdot X$ | 0.686        | $Y = 43.06 + 0.150 \cdot X$ | 0.876        |
| NaCl + PEPA    | $Y = 46.43 + 0.066 \cdot X$ | 0.668        | $Y = 52.67 + 0.102 \cdot X$ | 0.669        |
| Saturated NaCl solution | $Y = 57.72 + 0.105 \cdot X$ | 0.737        | –                          | –            |
| 2% ammonia solution | $Y = 41.01 + 0.144 \cdot X$ | 0.711        | –                          | –            |

Here, $Y$ is the average length of the seedling, mm; $X$ is the multiplicity of dilution.

The values of the correlation coefficients between the experimental data and the regression equations are much larger than their critical values [18]. This indicates a fairly good agreement between the experimental results and the regression equations obtained for their description. Therefore, the regression equations shown in Table 6 can be used to determine the safe dilution ratio. Thus, the safe dilution ratio obtained using Zabava variety was 554.8 for wastewater containing NH₃ + EDA and 892.9 for effluent containing NaCl + PEPA. The safe dilution rate for a 2% NH₃ solution makes 392.9, and
this one for a saturated NaCl solution makes 625.1. The safe dilution obtained using Krupnolistovoy variety was 489.1 for wastewater containing NH₃ + EDA and 545.8 for effluent containing NaCl + PEPA.

Table 7 shows the seedlings dry weight depending on the dilution ratio of wastewater containing NaCl + PEPA for each parallel experiment.

The dry weight of seedlings varies from 1.30 mg to 2.15 mg. The error determined for the averaged values of the dry weight of the shoots in each parallel experiment varies from 7.57% to 15.68%, which indicates a good reproducibility of this parameter. But the dependence of the dry weight of the seedlings on the effluent dilution ratio is not observed.

The regression equations and the correlation coefficients of the dependence of the dry weight of seedlings on the dilution ratio are shown in Table 8.

**Table 7.** Dependence of the seedlings dry weight on the dilution ratio of wastewater containing NaCl + PEPA.

| Dilution | Zabava Weight, mg | Error, % | Krupnolistovoy Weight, mg | Error, % |
|----------|------------------|----------|---------------------------|----------|
| 16       | 1.98             | 9.25     | 2.15                      | 10.03    |
|          | 1.86             | 10.13    | 1.96                      | 8.26     |
|          | 2.07             | 8.17     | 2.07                      | 9.48     |
| 32       | 1.96             | 8.87     | 1.88                      | 10.07    |
|          | 1.76             | 8.28     | 1.94                      | 9.01     |
|          | 1.85             | 9.51     | 1.95                      | 9.57     |
| 64       | 1.88             | 7.30     | 1.85                      | 9.68     |
|          | 1.78             | 8.83     | 1.66                      | 7.57     |
|          | 1.73             | 7.76     | 1.74                      | 7.98     |
| 128      | 1.48             | 7.53     | 1.52                      | 10.43    |
|          | 1.59             | 9.79     | 1.60                      | 9.48     |
|          | 1.57             | 8.35     | 1.53                      | 9.81     |
| 256      | 1.30             | 13.65    | 1.53                      | 10.07    |
|          | 1.38             | 9.79     | 1.64                      | 8.66     |
|          | 1.48             | 11.06    | 1.50                      | 11.60    |
| 512      | 1.49             | 6.90     | 1.44                      | 9.35     |
|          | 1.45             | 8.26     | 1.54                      | 11.68    |
|          | 1.41             | 11.77    | 1.49                      | 10.23    |
| 1024     | 1.51             | 9.78     | 1.64                      | 7.39     |
|          | 1.50             | 9.78     | 1.51                      | 10.16    |
|          | 1.58             | 6.75     | 1.45                      | 14.32    |
| Control  | 1.04             | 15.68    | 1.41                      | 9.09     |
|          | 1.25             | 12.53    | 1.34                      | 9.17     |
|          | 1.13             | 15.38    | 1.37                      | 10.36    |

**Table 8.** Regression equations and correlation coefficients of the dependence of the dry weight of seedlings on the degree of dilution.

| Effluent  | Zabava Regression equation | Zabava Correlation coefficient | Krupnolistovoy Regression equation | Krupnolistovoy Correlation coefficient |
|-----------|-----------------------------|--------------------------------|-----------------------------------|---------------------------------------|
| NaCl + PEPA | $Y = 1.677 + 0.00001\cdot X$ | 0.467                          | $Y = 1.733 + 0.00001\cdot X$      | 0.362                                 |

Here, $Y$ is the dry weight of the seedling, mg; $X$ is the multiplicity of dilution.

From table 8 it follows that the multipliers of the dilution ratio are quite low values. As a result, even with a 1000-fold dilution, the dry weight of seedlings varies from 0.6% to 5.7%. This indicates that the
dry weight of seedlings practically does not depend on the multiplicity of dilution of the flow for both watercress varieties. In this case, the correlation coefficients have values below their critical values [18]. All this suggests that these dependencies cannot be used to determine the safe multiplicity of wastewater dilution.

4. Conclusion

Studies show the acute toxicity of wastewater from ethylene diamine production. The germination of seeds of watercress for both varieties is observed, starting with a 16-fold dilution both for effluent containing NH₃ + EDA, and for effluent containing NaCl + PEPA. The obtained results state that to determine the safe dilution ratio of both effluents, only regression equations obtained from the dependence of the average seedling length on the dilution factor can be reliably used.

It should be noted that the effluent containing NaCl + PEPA is more toxic than the effluent containing NH₃ + EDA. At the same time, the Zabava variety of watercress turns out to be more sensitive to the waste water toxicity than the Krupnolistovoy variety. Thus, the safe degree of dilution of runoff containing NH₃ + EDA, determined by the length of seedlings of the Zabava variety, is almost 12% greater than the dilution ratio, found from the length of seedlings of the Krupnolistovoy variety of watercress. In the case of wastewater containing NaCl + PEPA, the same result makes almost 39%.

In conclusion, it can be noted that the Zabava and Krupnolistovoy varieties of watercress can be used to study the toxicity of wastewater containing various substances. The studies of Zabava varieties conducted with a saturated solution of NaCl and 2% NH₃ solution showed that the presence of impurities of polyethylene polyamines and ethylene diamine in these solutions increases its toxicity. However, to compare the toxicity of wastewater containing different components, it is necessary to use the same species and variety of plant as a phyto-indicator.

Acknowledgements

This scientific paper was prepared on basis of research work within the government work No. 5.12863.2018 / 8.9 of the Russian Federation.

References

[1] Kuliev A M 1985 Chemistry and technology of additives to oils and fuels (Leningrad: Khimiya)
[2] Varavskaya O A, Khvatov L K, Lebedev N A, Khlebnikov V N, Fakhrutdinov B R, Safin D H, Chebareva A I and V A Shepelin RU Patent 2,174,997 (20 October 2001)
[3] Levashov V I and Mudrik T P 2008 Bashkir Chemical Journal 15(4) 102-104
[4] Testova N V, Sukhova O B and Jonah K G RU Patent 2,114,849 (10 July 1998)
[5] Bykovsky N A, Daminev R R, Fatkullin R N and Puchkova L N RU Patent 2,226,189 (10 January 2015)
[6] Zagidullin R N RU Patent 2,537,564 (27 March 2004)
[7] Lebedev N N 1988 Chemistry and technology of basic organic and petrochemical synthesis (Moscow: Khimiya)
[8] Bykovsky N A, Puchkova L N and Fanakova N N 2015 Ecology and Industry of Russia 19(10) 48-51
[9] Daminev R R, Islamutdinova A A and Shayakhmetov A I 2012 Ecology of urbanized territories 2 80-84
[10] Melekhova O P and Yegorova E I 2010 Biological control of the environment: bioindication and biotesting (Moscow: Academia)
[11] Dmitriev A I 1996 Bioindication (Nizhny Novgorod: Volgo-Vyatsk Academy of Government Service)
[12] Evgenyev M I 1999 Soros Educational Journal [in Russian – Sorosovskiy obrazovatelnij zhurnal] 11 29–34
[13] Olkova A S 2014 Successes of modern biology [in Russian – Uspekhi sovremennoy biologii] 134(6) 614–622
[14] Persoone G 1992 Ecotoxicology and water quality standards *River water quality ecological assessment and control* (Brussels: Commission of European Communities) pp 461-482

[15] Braginsky L P 1977 *Problems of analytical chemistry* [in Russian – Problemy analiticheskoy khimii] 5 27-35

[16] Braginsky L P 1981 Water quality assessment of natural water bodies by toxicological indicators *Proc. Conf on Scientific bases of quality control of surface waters by hydrobiological indicators* (Moscow: Sovetskaya nauka) pp 201-206

[17] PND F T 14.1:2:4.19 2013 *Biological methods of control. Methods for determining the toxicity of drinking, groundwater, surface water and wastewater, chemical solutions by measuring the germination, average length and average dry weight of seedlings of watercress* (Moscow: Federal service for supervision of natural resources)

[18] Urbah V Yu 1964 *Biometric methods* (Moscow: Nauka)