Direct DNA interaction and genotoxic impact of three metals: Cadmium, nickel and aluminum

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are suspected to be involved in carcinogenesis which implies genomic
lesions. We propose to first study their genotoxic impact in vivo on
primary normal human dermal fibroblast (NHDF) cells with comet assay at
pH 7 to measure DNA breaks occurrence. Then, to characterize the
metal/DNA interaction by isothermal titration calorimetry (ITC).
Comet assay shows that Cd and Ni are genotoxic, they are responsible for
DNA breaks starting from $1.10-4$ mol.L-1 and $5.10-2$ mol.L-1, respectively
whereas Al has no effect (on DNA at pH7) as studied by ITC at pH 7. Cd
and Ni present an electrostatic interaction with DNA phosphate groups. At
high Cd concentration, a DNA condensation is observed by contrast. Al has
no interaction with DNA phosphate groups, but at pH 4 the electrostatic
interaction is strong and the same DNA condensation phenomenon is
observed. Metal genotoxic effect seems linked to the electrostatic
interaction on DNA phosphate groups. Genotoxic power evolves in parallel
to DNA phosphate interaction strength as Cd > Ni > Al. If this study
shows that metals do not directly break DNA, this binding could be a
preferential site for damage due to reactive oxygen species.

2 of November 2017

Dear Editor,

Please find herewith an original article exclusively submitted to Journal of Chemical Thermodynamics entitled "Direct DNA interaction and genotoxic impact of three metals: cadmium, nickel and aluminum". This article is complementary of a previous work published in Journal of Thermal Analysis and Calorimetry under the title "Effect of Ni(II), Cd(II) and Al(III) on human fibroblast bioenergetics, a preliminary comparative study".

The authors and I feel that this work merits publication as an article as it is the first time that the interaction between Cd^{2+}, Ni^{2+} and Al^{3+} with DNA is directly studied using a "non classic" model. This original thermodynamic study allows a fine approach of the mechanisms involved at the molecular level during binding of the metals on the DNA. Moreover, this study compares the thermodynamics of the interaction to the DNA damages observed by comet assay on living cells. In addition, this article emphasizes the contribution of the thermodynamics in the study of toxicology and environmental health.

The authors and I believe that the results of this study could usefully improve the comprehension of metal cations toxicity and could explain medical observations which remain not clearly understood at this time.

Please feel free to contact me for additional information.

Sincerely yours.

Dr. David Bergé-Lefranc

Highlights

- Nickel, cadmium and aluminum are ubiquitous metals known to induce human diseases.

- Anti-cooperative interaction between Cd^{2+}, Ni^{2+} and Al^{3+} with DNA

- In physiological conditions, cadmium and nickel present an electrostatic interaction with DNA phosphate groups whereas aluminum does not.

- Metal genotoxicity grows concomitantly to the intensity of the interaction with DNA phosphate groups.

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Abstract

This study simultaneously investigates direct DNA interaction and genotoxic impact of three typical metals: aluminum, cadmium and nickel, which the high concentration in soils and which the industries use, result in a daily significant exposure to humans. The three of them are suspected to be involved in carcinogenesis which implies genomic lesions. We propose to first study their genotoxic impact in vivo on primary normal human dermal fibroblast (NHDF) cells with comet assay at pH 7 to measure DNA breaks occurrence. Then, to characterize the metal/DNA interaction by isothermal titration calorimetry (ITC).

Comet assay shows that Cd and Ni are genotoxic, they are responsible for DNA breaks starting from 1.10^{-4} mol.L^{-1} and 5.10^{-2} mol.L^{-1}, respectively whereas Al has no effect (on DNA at pH7) as studied by ITC at pH 7. Cd and Ni present an electrostatic interaction with DNA phosphate groups. At high Cd concentration, a DNA condensation is observed by contrast. Al has no interaction with DNA phosphate groups, but at pH 4 the electrostatic interaction is strong and the same DNA condensation phenomenon is observed. Metal genotoxic effect seems linked to the electrostatic interaction on DNA phosphate groups. Genotoxic power evolves in parallel to DNA phosphate interaction strength as Cd > Ni > Al. If this study shows that metals do not directly break DNA, this binding could be a preferential site for damage due to reactive oxygen species.

Key words: aluminum; cadmium; nickel; isothermal titration calorimetry; genotoxicity; DNA binding

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1. Introduction

This study examines three metal, aluminum, cadmium and nickel, (which the high concentration in soils and which the industries use result in a daily significant exposure to humans. If toxicity of Cd and Ni is clearly established and demonstrate, it is not the case of Al whose effects are still controversial. Indeed, chronic exposure to Cd leads to tubular and glomerular dysfunction and then to renal insufficiency accompanied by bone disorders [1-3]. Since 1973, Cd is considered as responsible for professional diseases and has been classified in group 1 carcinogenic agent by the IARC [4]. In the same way, Ni, which we handle daily with coins, jewelry or zippers, is recognized as one of the most allergenic metals [5, 6]. Moreover, besides leading to contact dermatitis, this metal is also classified as a potentially carcinogenic substance for humans by the IARC [7] and a chronic exposure to nickel is registered as a risk factor for lung cancer in the professional illness table. Finally, Al is said to be responsible for dialysis encephalopathy, osteoporosis and osteomalacia [8]. It accumulates in bones and brain leading to memory and learning disorders, but also inducing neurodegenerative pathologies [9-11]. If its role in breast cancer has been mentioned [12, 13] but not clearly established, according to precautionary principle its action as a carcinogen cannot be totally invalidated.

However, if the entire mechanism of action of these metals remains mostly unknown, their carcinogenesis abilities cannot be ignored. In this condition, it is relevant to consider their impact on the genome that, when altered, may lead to tumors. The aim of this study is to determine if these metals have an impact on the genome in physiological conditions. Then, if so, to quantify and model this impact. So, at first, the impact on Al, Cd and Ni on the genome was evaluated on human cells by comets assay. Secondly, the chemical interaction between metals and DNA was defined, using isothermal titration calorimetry (ITC), then the interaction was studied by the McGhee von Hippel model [14].

2. Materials and methods

2.1 Reagents

The metal used were chlorides salts at high purity (99.9%): Cd (II) and Ni (II) were from Aldrich® and Al (III) from Fluka® analytical.

Primary normal human dermal fibroblasts (NHDF) were purchased from Lonza®. Fibroblast basal medium-2 (FBM-2) supplemented with a growth kit containing 10 mL of fetal bovine serum, 0.5 mL of insulin, 0.5 mL of gentamicin sulfate amphotericin-B (GA-1000), and 0.5

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mL of r-human fibroblast growth factor-B were also from Lonza®. During the harvest, NHDF were treated by Trypsine-EDTA (1x) 0.05% purchased from Gibco®.

Sodium chloride (NaCl) used in aqueous solution was from Sigma-Aldrich® and ≥ 99.5% of purity.

The TRIS (2-amino-2-hydroxyméthyl-1,3-propanediol) and MES (2-(N-Morpholino)ethanesulfonic acid) buffers were from AppliChem of Biochemical Chemical Synthesis Services and from Sigma-Aldrich® and ≥ 99.5% of purity (titration) respectively.

The DNA used in thermal analysis experiment was low molecule weight from salmon sperm from Sigma-Aldrich®.

Low Melting Point and Normal melting Point agars, L-lauroyl sarcosinate, dimethylsulfoxide, Triton X-100, ethidium bromide and methanol of analytical grade were from Sigma-Aldrich®

2.2 Cell culture

Fibroblasts were routinely cultured in culture medium (FBM-2) in sterile boxes for cell culture with an area of 75 cm^2 (T75) and maintained at 37°C and 5% CO_2. When cells were at confluence, FBM-2 was discarded and cells were treated with the trypsin solution during 2 minutes at 37°C. The cell suspension obtained was diluted in FBM-2 and introduced into new T75 boxes. NHDF were then used for all experiments between passages 5 and 8.

2.3 Comet assay

The comet assay performed was the alkaline comet assay as described by Singh [15]. NHDF were harvested, put in a 24 multi-wells plate to get 160 000 cells per well and incubated during 24 hours before treatment. At the end of the 2 hours exposure period with a concentration of Cd ranges between 50 and 500 µmol.L^{-1}, Ni from 5 to 50 mmol.L^{-1} and Al from 1 to 10 mmol.L^{-1}, cells were trypsinized with 0.05% trypsin-0.02% EDTA in PBS for 3–5 minutes at 37°C, and centrifuged at 1200 rpm for 5 minutes. Following the trypsin/EDTA treatment, cells were mixed with PBS and centrifuged at 1200 rpm for 5 minutes. During cell centrifugation, a first layer of 85µl of 0.8% NMP agarose (in PBS) was deposited on microscope slides, previously coated with 2% NMP agarose (in PBS) and allowed to dry for 12 hours. Coverslips were placed over this layer and the agarose was allowed to harden during 5 minutes on ice. After centrifugation coverslips were removed, cell pellets were mixed with 75µl of 0.5% LMP agarose (in PBS) and deposited onto the microscope slides. Two slides were prepared for each sample. Coverslips were placed over the cell layer and the agarose was allowed to harden during 5 minutes on ice. Then, coverslips were removed, and a

third layer with 85µl of 0.8% agarose (in PBS) was added over the cells. A coverslip was applied and the agarose was allowed to solidify during 5 minutes on ice.

The slides were immersed in freshly prepared lysis solution (2.5 M NaCl, 100 mM Na2 EDTA, 10 mM Tris (pH 10), 1% L-lauroyl sarcosinate, with 10% dimethylsulfoxide and 1% Triton X-100 added just before use) for 90 min at 4°C. After the lysis procedure, the slides were placed side-by-side in a horizontal gel electrophoresis tank, which was filled with fresh electrophoresis buffer (300 mM NaOH, 1mM Na2 EDTA pH >13). The slides remained in the buffer for 20 minutes at 25°C to allow DNA unwinding. Electrophoresis was then carried out in the same buffer for 20 minutes at 25 V and 300 mA. Following electrophoresis, the slides were washed three times for 5 minutes each with 0.4 M Tris buffer (pH 7.5) to neutralize the alkali. Finally, the slides were rinsed with ultra-pure water, dehydrated in 100% methanol and dried for 12 hours at room temperature. Each slide was stained with 50µl of 2 µg/ml ethidium bromide and covered with a coverslip.

The cells were examined at 250X Magnification using a BX53-RFL fluorescence microscope (Olympus Optical Co. Tokyo, Japan) equipped with a U-MWG2 dichroic mirror (band-pass filter, 510-550 nm; long-pass filter, 590 nm) and a UPLFLN 20x objective. Image analysis was performed using the Komet software (version 6.0 Andor Technology, Belfast, Northern Ireland). A total of 50 randomly selected cells were analyzed per slide using Fenestra Komet 6.0 image analysis software Andor Technology (Belfast, Northern Ireland). DNA damage was expressed as the Olive Tail Moment (OTM; arbitrary units); 100 OTM values were determined for each sample, 50 from each of two separate slides/sample. The 100 calculated OTM values by sample were distributed into 30 classes between the minimal and the maximal values: each class interval corresponded to 1.4 OTM arbitrary units. A non-linear regression analysis was performed on the OTM distribution frequencies by using a χ^2 function with Table Curve 2D software (version 5.0; Jandel Scientific Software, San Rafael, CA). The calculated degrees of freedom (n) for this function were quantitative measures of the DNA damage for a sample [16, 17]. The n was termed χ^2 OTM and was used as the sole parameter for assessing levels of DNA damage. The significance of the differences between χ^2 OTM values of control and treated cells was analysed using Student's t-test.

2.4 Calorimetry

Isothermal Titration Calorimetry (ITC) was performed on a Microcal ITC 200 instrument (Microcal, Northampton, United States) at 37°C unless otherwise specified in TRIS (5mM and pH = 7) or MES (5mM and pH = 4) buffer. The metal solution (8mM for Al and 10 mM

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for Cd and Ni) was added sequentially after an initial injection of 0.1 µL (not used in data fitting) [18] by adding 30 injections of 1.3 µL at 90s of intervals. The measuring cell contained a DNA solution at the initial concentration of 2 mmol.L^{-1} of phosphate groups, solubilized in the same buffer and at the same pH, than the metal solution. These concentrations (*a priori* high) were chosen in order to obtain the most accurate heat flow and do not change the parameters and the thermodynamics of the interaction. The reference power and the feedback gain were modified according to the thermal power of peaks. Each experiment was performed three times. Peaks were integrated and heats of dilution subtracted, yielding the heat values of each injections.

The binding between metal cations and phosphate groups of DNA were studied using McGhee von Hippel model [19]. DNA is considered as a mono-dimensional lattice with n sites *i.e.* phosphates. Each metal cation during the binding blocks the accessibility to L number of phosphates. Along the DNA saturation, the number of metal cations bound per nucleotide is defined as:

$$\theta = \frac{[M^{L+}]}{[P^-]_t}, \tag{1}$$

Here $[M^{L+}]$ and $[P^-]_t$ represent the concentration of metal cation bound and the total concentration of DNA phosphate groups, respectively. Considering the ionic interaction, the θ value is ranged between 0 and n/L. If infinite lattice is considered and end-effects disregarded, the theoretical isotherms of McGhee von Hippel [14] is obtained:

$$\frac{\theta}{[M^{L+}]_f} = K.(n - L.\theta).\left(\frac{n - L.\theta}{n - (L-1).\theta}\right)^{(L-1)} \tag{2}$$

Here variables are defined as in (1), $[M^{L+}]_f$ is the free metal cation concentration, K is the binding constant and n is the number of repeating units per DNA (in our case the value is equal to 1 due to the consideration of DNA phosphate group concentration).

For L > 1, an apparent negative cooperation between ligands occurs during binding frequently called entropic resistance to saturation. In the ionic interaction, L can represent the size but also the charge of metal cation. The binding polynomial can be written as:

$$([M^{L+}]_t - \theta.[P^-]_t).(n - L.\theta)^L - \frac{\theta.(n - \theta.(L-1))^{(L-1)}}{K} = 0 \tag{3}$$

The polynomial obtained possesses only one root presenting physical meaning *i.e.* being superior to zero and lower than 1/L.

The determination of the root was performed using a library of numerical routines from NAG (Numerical Algorithms Group, Inc) included in Origin 9.1. The NAG function c05tbc is a

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function which finds a non-linear system equations solution by a modification of the Powell hybrid method [20]. The iteration procedure was continued until both chi-square tolerance value and coefficient of determination values equal to 10^{-9} and 0.995 were respectively reached.

For each injection, considering the mass balance and the cell volume, the total concentration of each reactant in the cell is calculated. From these concentrations, the iteration process leads to the determination of L, K and θ. Finally, the heat effect for each injection q_i is calculated from:

$$q_i = \frac{\partial \theta}{\partial R} \qquad (4)$$

where R represent the ratio $[M^{L+}]_t \big/ [P^-]_t$.

The use of Levenberg-Marquardt algorithm in a nonlinear curve fitting procedure allows the determination of L, K and ΔH° values from the experimental data.

3. Results

3.1 Comet assay

Comet assay was performed in order to measure the induction of DNA-strand breaks by metals.

As comet assay was significant by using metal concentrations lower than those inducing cytotoxicity, the value was determined by MTT assay at 2h. Then IC50 was found equal to 1.10^{-3}M, 3.10^{-3}M and 4.10^{-3}M for Cd, Ni and Al respectively (data not shown).

Cadmium and nickel induced a significant increase of DNA-lesions, while aluminium did not exert genotoxic activity. Cadmium induced the most important DNA damage with a significant induction of comets at 100 µmol.L^{-1} (fig.1). Nickel appeared far less active, with comets significantly present at 50 mmol.L^{-1} (p < 0.001) (fig.2), a concentration which is about 10^6 over blood concentration [21] (Ivanenko et al., 2012). Aluminium was not responsible of any damaging impact on DNA at concentrations tested (fig.3).
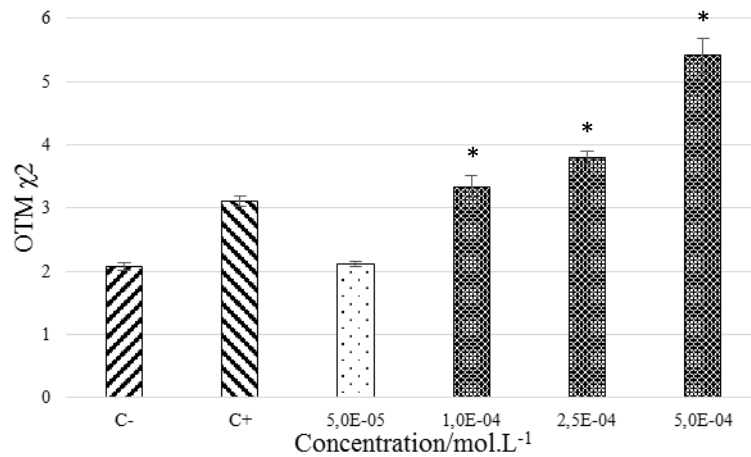
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Figure 1: Cadmium impact on DNA. The first bar is the positive control. Significant assay are represented by the dark bars with stars (p<0.001)
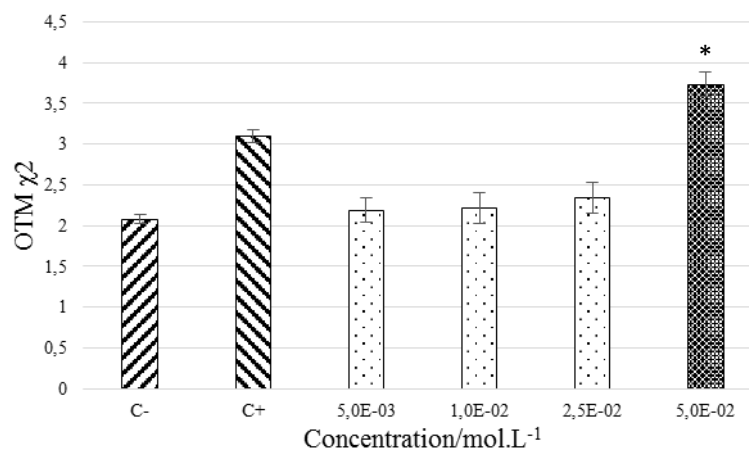

Figure 2: Nickel impact on DNA. The first bar is the positive control. Significant assay is represented by the dark bar with the star (p<0.001)
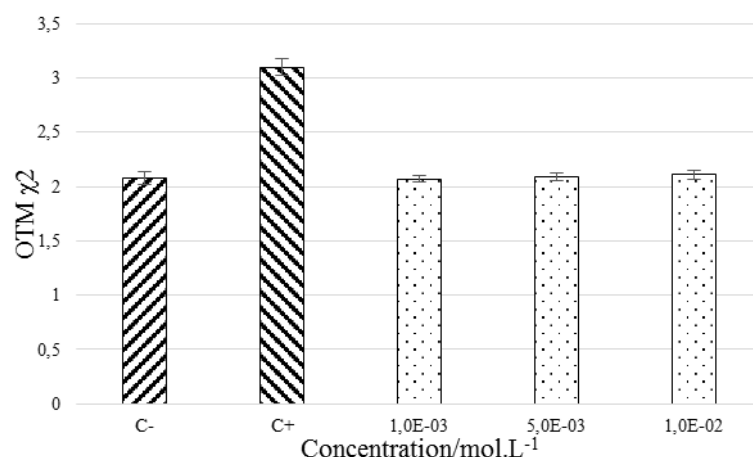
Figure 3: Aluminum impact on DNA. The first bar is the positive control. No significant result

The genotoxic impacts of cadmium and nickel confirmed previously published data [22, 23], while the absence of genotoxicity of aluminum was also demonstrated [24]. However, the strength of the toxic action that occurred is different. Indeed, cadmium acts at lower concentrations than nickel, 1.10^{-4} versus 5.10^{-2} mol.L^{-1} respectively.

Over 4.10^{-5} mol.L^{-1}, at pH 7 and under physiological condition in aqueous medium at 0.15 mol.L^{-1} NaCl, aluminum is not present as a free trivalent cation (Al^{3+}) but under different complexes as illustrated in (fig. 4) (only monomolecular complexes are considered) [25-27]. The lack of genotoxic effect may be explained considering that at pH equal to 7 the Al^{3+} specie is negligible and the major fraction (more than 95%) of aluminum is under [Al(OH)$_4$]$^-$ form.
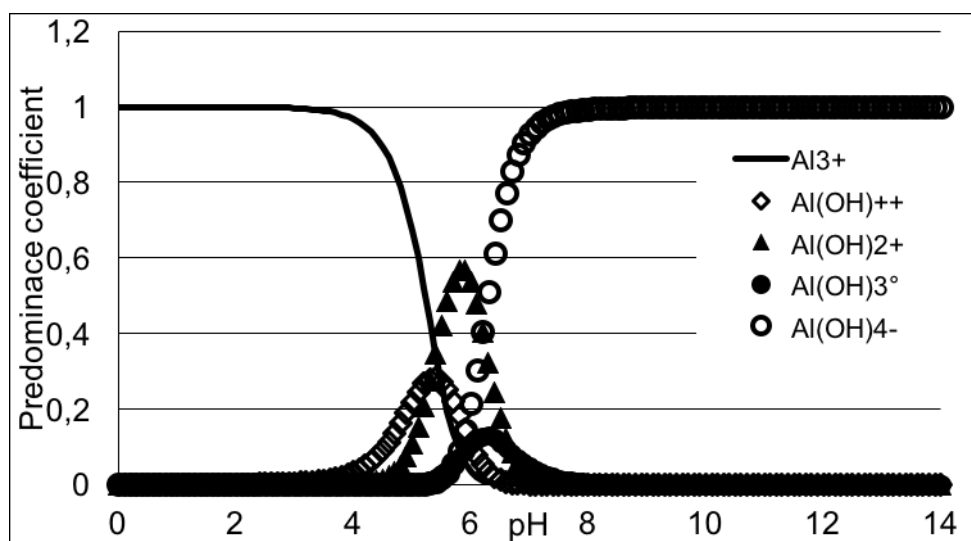

Figure 4: Species predominance diagram of aluminum in water at NaCl concentration = 0.150 mol.L^{-1}

3.2 Isothermal titration calorimetry

At first, interaction between each metal and DNA are performed at constant ionic strength obtained with a NaCl solution (0.150 mol.L^{-1}) and pH 7 in order to mimic the physiological conditions, Al was tested also at pH 4. Results obtained for interactions are given in Table 1.

Metal	pH	L	K/L.mol^{-1}	$\Delta H°$/kJ.mol^{-1}	R^2
Cd^{2+}	7	6.7 ± 0.4	57 ± 4	-24.4 ± 1.2	0.993
Ni^{2+}	7	6.5 ± 0.2	26 ± 3	-20.3 ± 0.9	0.999
Al^{3+}	7	NB[a]	NB	NB	NB
Al^{3+}	4	3.4 ± 0.1	2021 ± 57	8.4 ± 0.4	0.999

Table 1: Thermodynamic Parameters of Interaction at 37°C, (pH = 7 in TRIS buffer or pH = 4 in MES buffer) and NaCl concentration of 0.150 mol.L^{-1}

[a]NB = not bound

The non-specific electrostatic binding of the model is validated by the good values of the coefficient of determination (R^2). The enthalpy driven of the process confirms that the binding is driven by an electrostatic interaction between the negative charge of the DNA phosphate groups and the positive charge of metal cation. The binding of metal cation on the phosphate groups decreases the charge density of DNA and then diminishes the affinity of Ni^{2+} and Cd^{2+} toward DNA. The interaction intensity evolves with metal concentration. Indeed, affinity is high at weak cation concentration and decreases when concentration increases (due to the DNA charge density diminution). This nature of the interaction excludes the validity for using a single independent site model and may lead to thermodynamic parameters without sense.

In these experimental conditions, divalent cations exhibit similar thermodynamic parameters with a binding constant and enthalpy slightly more favorable to Cd^{2+} than Ni^{2+}. This stronger affinity of Cd^{2+} to phosphate groups, can be related to the weaker solubility of Cd$_3$(PO$_4$)$_2$ than Ni$_3$(PO$_4$)$_2$. Moreover, this difference of behavior on the affinity to DNA was observed in a previous study of the interaction between Cd^{2+} and Ni^{2+} on DNA helicity [28]. At pH 7, no binding can be observed with aluminum, which confirm Wu et al. [29] observations performed at pH = 6. As said above, in acid conditions aluminum is present under Al^{3+} specie, therefore interaction experiments were performed at pH = 4 in order to compare the interaction of Al^{3+} to Cd^{2+} and Ni^{2+} (fig.5). This result clearly shows that from a chemical point of view, aluminum interacts with DNA only under cationic form. The interaction between Al^{3+} and DNA is largely entropy driven coupled with an unfavorable enthalpy. This

calorimetric signature and the pH range of binding were already mentioned in a previous study using a single set of identical sites model [29]. This strong interaction of Al^{3+} with phosphates is well known and frequently met in biological systems but the variation of the affinity during the binding is not [30, 31]. The electrostatic component of the interaction is in agreement with DFT quantum mechanical calculations describing the phosphate of DNA as strong chelators [32, 33].
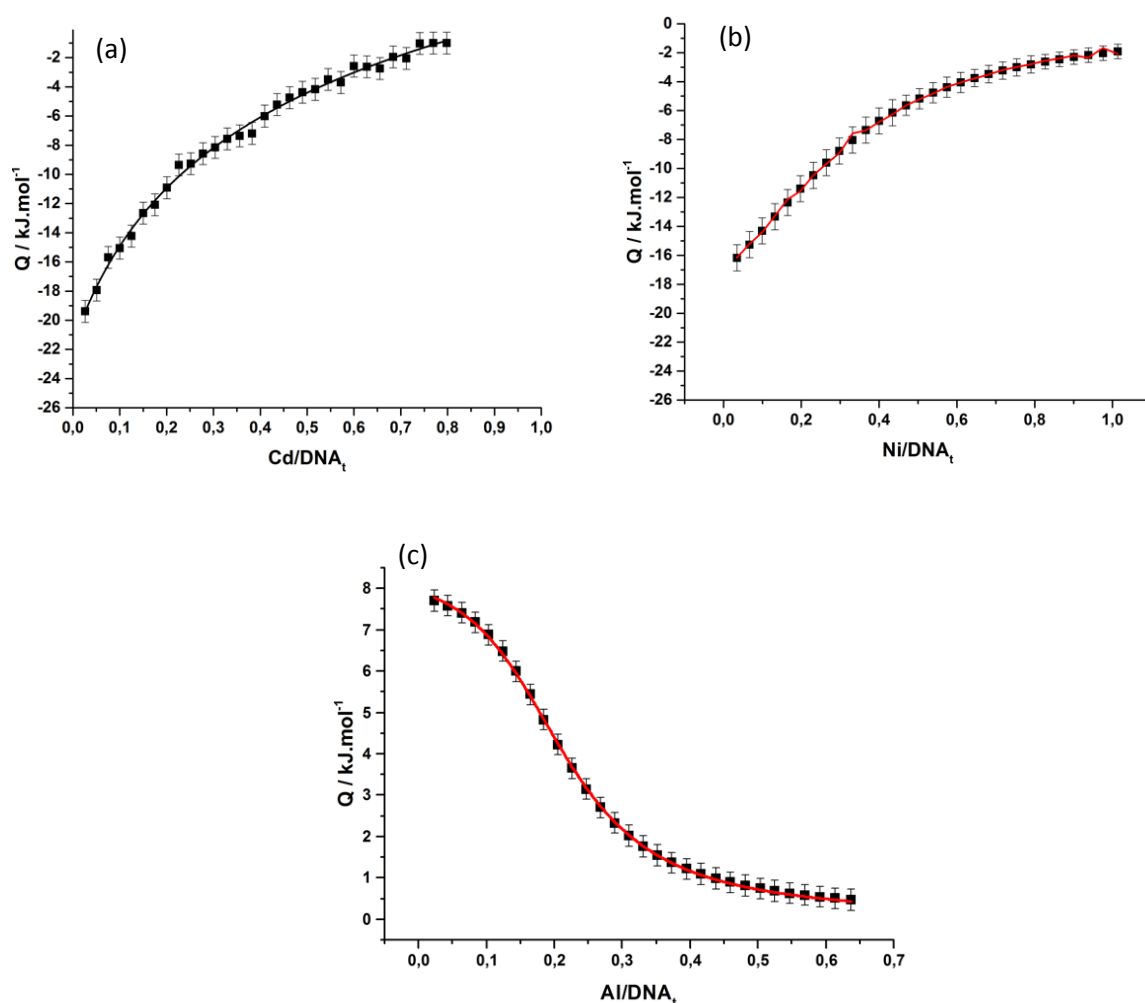

Figure 5: (a) Isothermal titration curves of Cd^{2+}, at 37°C at 0.150 mol.L-1 NaCl in TRIS buffer at pH 7. (b) Isothermal titration curves of Ni^{2+}, at 37°C at 0.150 mol.L-1 NaCl in TRIS buffer at pH 7. (c) Isothermal titration curves of Al^{3+}, at 37°C at 0.150 mol.L-1 NaCl in MES buffer at pH 4.
Error bars correspond to the mean standard error.

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Ionic strength influence

As electrostatic interaction and binding constant are strongly dependent of the ionic strength, experiments are performed next at different NaCl concentrations [34, 35]. Indeed, the electrostatic component of the interaction depends on the salt concentration and on the non-electrostatic component, as described by the following equation [36]:

$$\ln(K) = \ln(K_{NE}) - n . \ln[NaCl] \qquad (5)$$

here K_{NE} represents the binding constant of the non-electrostatic component of the interaction, n is the number of counter-ions displaced from the phosphate groups of DNA and [NaCl] is the salt concentration.

Ionic strength is then decreased by diminishing the NaCl concentrations at pH = 7 for Cd^{2+} and Ni^{2+}. In the Al^{3+} case, pH is fixed at 4 to isolate the trivalent specie aluminum. The affinity constant evolution versus the sodium chloride concentration is plotted in figure 6. The constant affinity at value weak ionic strength can only be measured on Ni^{2+} titration. Indeed, in the case of Cd^{2+} a condensation of DNA occurred. This behavior is often observed when a critical concentration of cation is reached. The DNA phosphate groups involve a charge density equal to - 0,4712 nC.m^{-1} (two negative charges per 0.34 nm). When metal cation is added, the negative charges of DNA backbones are neutralized and lead to the condensation and aggregation of DNA (the phenomenon is easily observed due to the turbidity occurring in the sample cell) [37, 38]. This DNA condensation during Cd^{2+} titration can be observed in figure 7. Since a value of Cd^{2+}/DNA-P about 0.2 is reached, a large endothermic peak appears due to DNA condensation. This behavior does not allow an usable fit of the titration curve. This value of 0.2 has already been observed in a previous electrophoresis study mentioning a Cd^{2+}/DNA stoichiometry of 1/5 i.e. 0.2 [39]. Once again this condensation process occurs only with Cd^{2+}, which confirms the strongest interaction of Cd^{2+} versus Ni^{2+}. This absence of transformations in Ni^{2+} interaction case is in accordance with UV spectroscopic investigations [40].
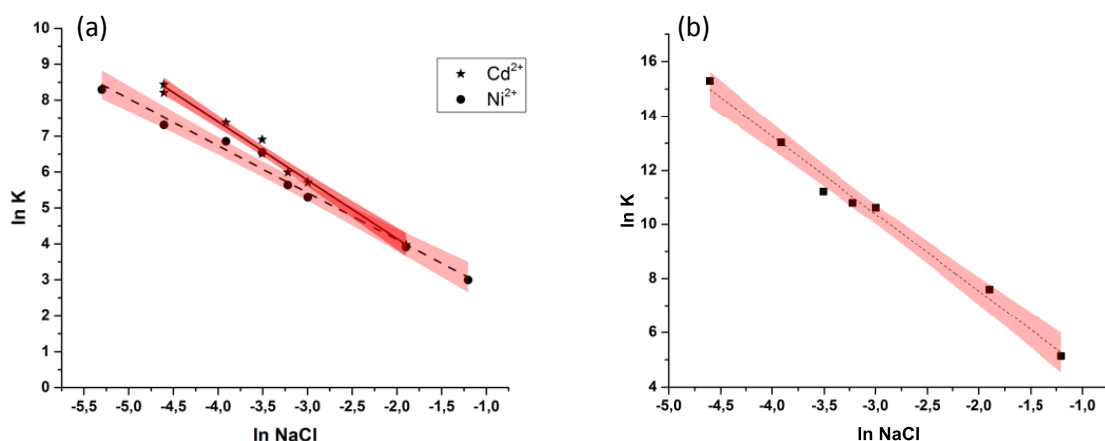
Figure 6: Dependence of the affinity constant on sodium chloride concentration. (a) Dashed and solid lines represent the linear fitting for Cd^{2+} and Ni^{2+}. (b) Dashed and solid lines represent the linear fitting for Al^{3+}.The shade bands around the linear fits represent the confidence interval of 95%.
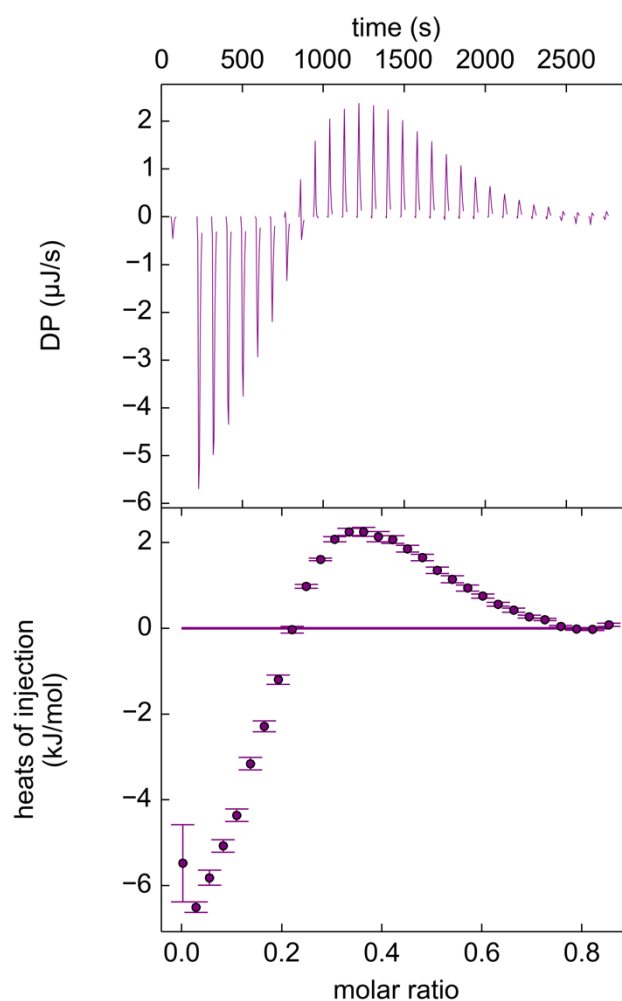
Figure 7: Isothermal titration curve of Cd2+ binding to DNA at 37°C in TRIS buffer at 5 mmol.L-1 with NaCl concentration lower than 5 mM. The upper curve shows the heat flow versus the time and the lower part shows the heat versus the Cd2+/DNA-P molar ratio [41].

Interaction mechanism

The natural logarithm plot of the affinity constant versus the natural logarithm of NaCl concentration exhibits a linear evolution associated with coefficients of determination (R^2) equal to 0.988, 0.983 and 0.989 for Ni^{2+}, Cd^{2+} (fig. 6.A) and Al^{3+} (fig. 6.B), respectively. This behavior is characteristic of the coulombic component governing the interaction. Indeed, Al^{3+}, Cd^{2+} and Ni^{2+} need to displace Na^+ to bind DNA phosphate groups. The increase of ionic strength involves a less favorable electrostatic entropy leading to a weaker binding. This is observed in high NaCl concentration than at low [42]. In the Al^{3+} case, the slope value is equal to 2.86 ± 0.13, leading to the displacement of 2.86 charges to bind one Al^{3+} ion. This displacement of counter-ions by aluminum is well known and can occur even with divalent counter-ions such as Mg^{2+} [43]. The amounts of counter-ions displaced by binding of Cd^{2+} and Ni^{2+} is equal to 1.63 ± 0.07 and 1.38 ± 0.07 respectively, confirming the strongest affinity

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of Cd^{2+} versus Ni^{2+} to DNA. These values of exchanged counter-ions are in the range of the ones observed during DNA and cationic ligand interaction, about 0.88 z (z represents the ligand valence) [44-46].

4. Discussion

Comet assay showed than cadmium and nickel had an impact on DNA while aluminum did not, at pH 7. These observations were congruent with thermal analysis results. Indeed, the most damaging metal was Cd^{2+}, according to comet assay, and it was also the strongest linked to DNA. Ni^{2+} had an intermediate effect in both experiments, and aluminum did not damaged DNA in comet assay and was not linked to DNA phosphates. This may be explained by considering that at pH 7 aluminum is mainly present under $[Al(OH)_4]^-$ form. To obtain Al^{3+} the pH value must be lower than 4 but these acidic conditions are responsible for cytotoxicity, mutagenic and clastogenic effects on cells [47, 48]. In this condition, legitimate questions can be raised about studies which find genotoxic effects over 40 $\mu mol.L^{-1}$ of Al^{3+} concentrations. If pH is kept at pH 7 (like in our study), Al^{3+} is no longer involved while other species, not entirely soluble, are present. According to comet results, no "tank effect" with aluminum appeared; undissolved aluminum amount did not impact DNA, there were no breaks. Over 40 $\mu mol.L^{-1}$, Al^{3+} should not be the specie tested.

According to these experiments, the genotoxicity of these metals seemed to be related to DNA phosphates: the strongest metal linked (Cd^{2+}) was also the most genotoxic. At the same time, the less bounded (aluminum) was the less toxic. However, these experiments confirm that the genotoxic effect of Cd^{2+} and Ni^{2+} is indirect [49]. Indeed, despite the high energy value of the bond, no heat peaks of thermal events have been measured excluding the possibility of direct DNA break.

Metals are known to act indirectly by generating reactive oxygen species (ROS) [50-52] which are responsible for DNA base oxidation resulting in DNA breaks. Metal linked to DNA phosphate groups could modify DNA electric charge repartition and ease ROS access until bases. Furthermore, the condensation effect observed with Cd^{2+} means that this metal has an impact on DNA conformation which could lead, after too much tension, to DNA break.

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5. Conclusions

Two metals are responsible for a genotoxic effect under physiological conditions: cadmium and nickel. These two, in the same condition, present an electrostatic interaction between the positive cation metal charge and the negatively charged DNA phosphate groups, influenced by ionic strength. It appears that the most genotoxic metal (cadmium) is also the most bound metal to phosphate groups. Its interaction is so strong that a DNA condensation happens when every negative phosphate charges are saturated at high metal concentration. Furthermore, the strength of the interaction is highlighted by the amounts of displaced counter-ions: 1.63 and 1.38 for Cd^{2+} and Ni^{2+}, respectively. Differences in genotoxicity of Cd and Ni may be due to the interaction of the metal with DNA. Indeed, Cd induces a stronger change on DNA electric field than Ni. This phenomenon might play a role by facilitating the effect of other active compounds such as ROS. For aluminum, the interaction with DNA is strong at pH 4 and not observed at pH 7. As at these pH values the main species of this metal is Al^{3+} and $[Al(OH)_4]^-$ respectively, solely Al^{3+} specie is able to bind strongly with phosphate groups of DNA as Cd^{2+} and Ni^{2+}.

This behavior highlights that more than the metal nature itself, its specie may be considered to be involved in the genotoxicity. In this way a role of intracellular pH may be emphasized, a change in this pH range may induce a change of the metal species and so of its distribution and action.

'Conflicts of interest: none'

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Table1

Metal	pH	L	K/L.mol^{-1}	ΔH°/kJ.mol^{-1}	R^2
Cd^{2+}	7	6.7 ± 0.4	57 ± 4	-24.4 ± 1.2	0.993
Ni^{2+}	7	6.5 ± 0.2	26 ± 3	-20.3 ± 0.9	0.999
Al^{3+}	7	NB^a	NB	NB	NB
Al^{3+}	4	3.4 ± 0.1	2021 ± 57	8.4 ± 0.4	0.999

Table 1: Thermodynamic Parameters of Interaction at 37°C, (pH = 7 in TRIS buffer or pH = 4 in MES buffer) and NaCl concentration of 0.150 mol.L^{-1}
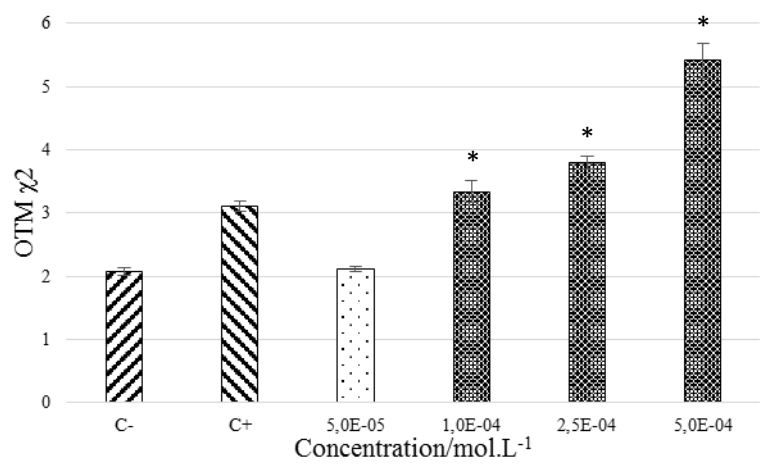
^{a}NB = not bound

Figure1

Figure 1: Cadmium impact on DNA. The first bar is the positive control. Significant assay are represented by the dark bars with stars (p<0.001)
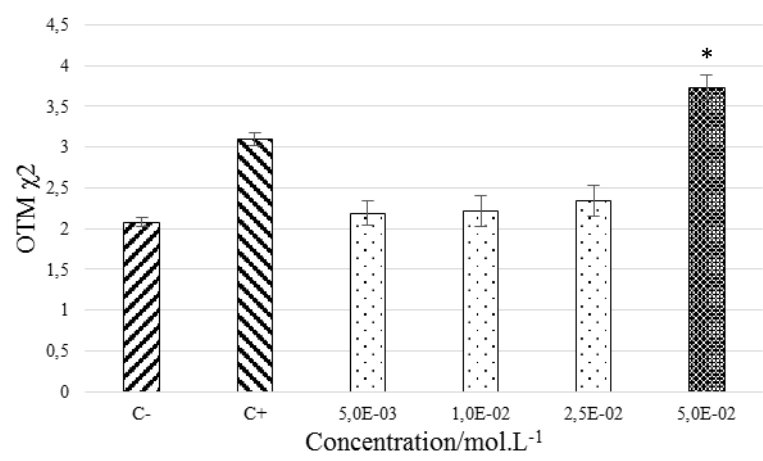
Figure2

Figure 1: Nickel impact on DNA. The first bar is the positive control. Significant assay is represented by the dark bar with the star ($p<0.001$)
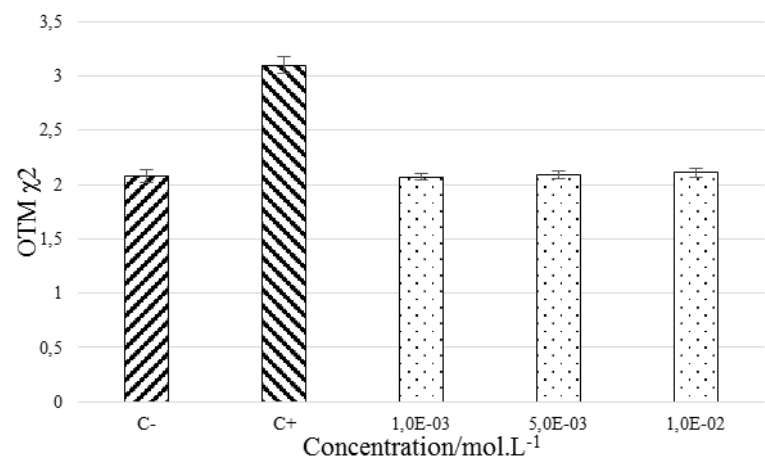
Figure3

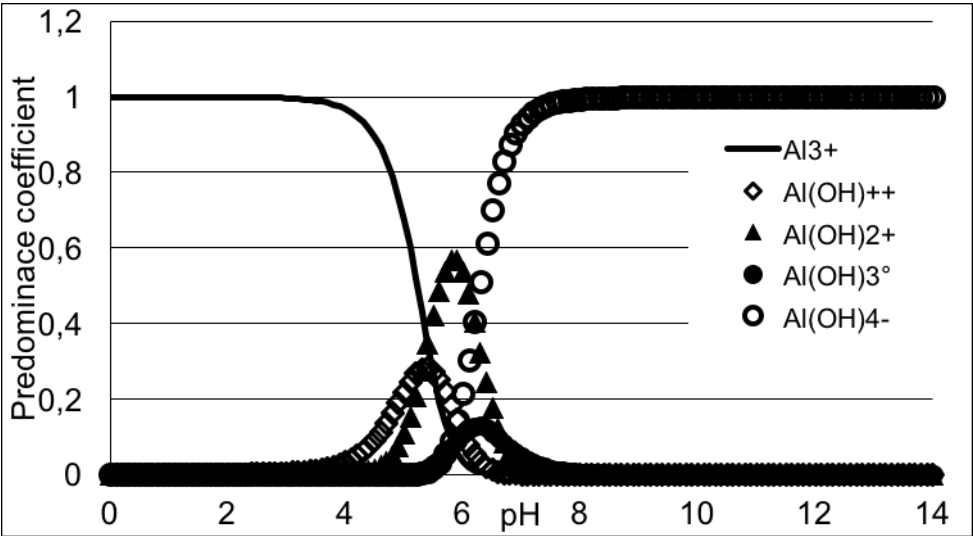*Figure 1: Aluminum impact on DNA. The first bar is the positive control. No significant result*

Figure4

Figure 1: Species predominance diagram of aluminum in water at NaCl concentration = 0.150 mol.L^{-1}
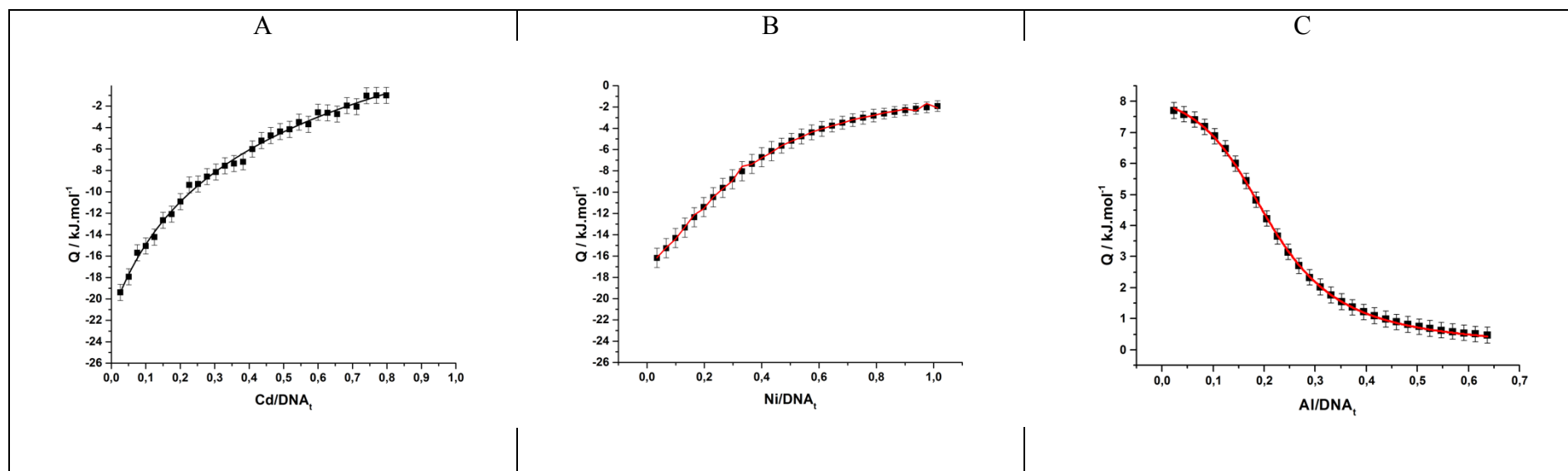
Figure5

Figure 1: (A) Isothermal titration curves of Cd^{2+}, at 37°C at 0.150 mol.L-1 NaCl in TRIS buffer at pH 7. (B) Isothermal titration curves of Ni^{2+}, at 37°C at 0.150 mol.L-1 NaCl in TRIS buffer at pH 7. (C) Isothermal titration curves of Al^{3+}, at 37°C at 0.150 mol.L-1 NaCl in MES buffer at pH 4. Error bars correspond to the mean standard error.
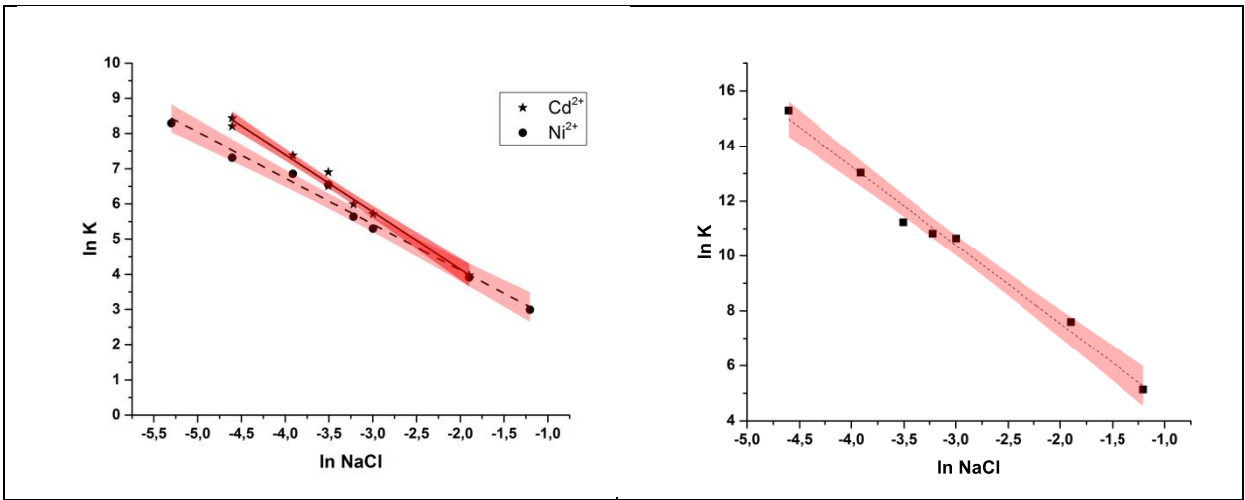
Figure6

Figure 1: Dependence of the affinity constant on sodium chloride concentration. (a) Dashed and solid lines represent the linear fitting for Cd^{2+} and Ni^{2+}. (b) Dashed and solid lines represent the linear fitting for Al^{3+}. The shade bands around the linear fits represent the confidence interval of 95%.

Figure7

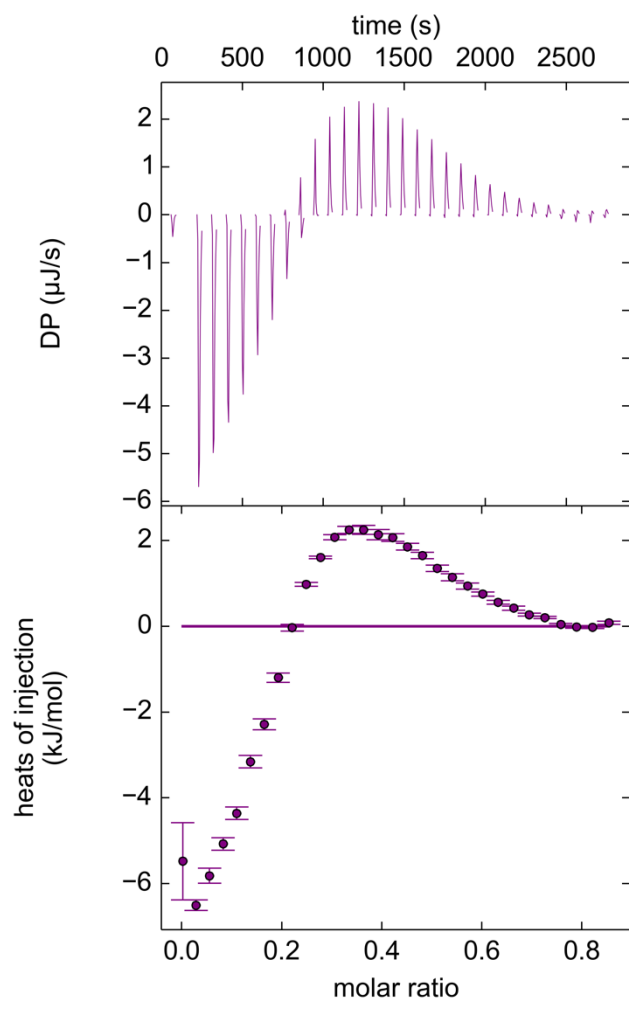*Figure 1: Isothermal titration curve of Cd2+ binding to DNA at 37°C in TRIS buffer at 5 mmol.L-1 with NaCl concentration lower than 5 mM. The upper curve shows the heat flow versus the time and the lower part shows the heat versus the Cd2+/DNA-P molar ratio [41].*