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DETERMINATION OF ZINC AND COPPER IN FISH SAMPLES COLLECTED FROM EGYPT RIVER NILE BY DIFFERENTIAL PULSE ANODIC STRIPPING VOLTAMMETRY

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ABSTRACT:

Zinc and copper contents in the edible parts (muscle, fillet) of 17 commercially used fish species from South Egypt River Nile (Aswan) were determined by means of DPASV (differential pulse anodic stripping voltammetry) at Hanging Mercury Drop Electrode (HMDE). In the sample preparation step, all fish samples were lyophilised, milled in a ball mill and finally decomposed by using mixed acid (HNO_3+HClO_4). The accuracy of the concentrations determined in this study was checked by the measurements of the certified reference material CRM No. 422, cod muscle from the Commission of the European Communities, Community Bureau of Reference. All Zn and Cu concentrations observed from species of Egypt River Nile showed that fish from this area is a good source of these essential elements and the developed method is accepted as a good analytical routine method for these samples. The great advantage of DPASV is the simplicity, selectivity, sensitivity, and shortening analysis time over the Atomic Absorption spectrophotometric AAS method.

INTRODUCTION:

Zinc and copper are essential elements for human beings[20-25] which means that they must be a part of our diet. However, these elements also can be toxic at high concentrations. There are numerous sample treatment procedures[16,21,26] and several analytical methods described for the determination of these trace elements[4, 11-14, 22, 27]. Zinc is found in almost every cell and in a wide variety of foods. It is present in seafood in mg/kg amounts and there have been no reports of concentrations in the edible parts of food fish that form a hazard to health. With an average zinc content of 3–5 mg/kg wet fish, it is a good source for this essential element[17-19]. The essential role of zinc is based on its roles as an integral part of a number of metalloenzymes and as a catalyst for regulating the activity of specific zinc dependent enzymes. Molluscs contain the greatest concentration of zinc. Among all food sources of animal origin, oyster is the richest source of zinc. Recorded values in classostreid oysters exceed 4000 mg/kg of dry weight[9].

Copper is required for iron utilization, and as a cofactor for enzymes involved in glucose metabolism and the synthesis of hemoglobin, connective tissue and phospholipids. Numerous studies have focused on copper metabolism in fish and on toxic effects related to heavy metal pollution in the aquatic environment[9];

however, it is not toxic for humans in low concentrations[10, 24].

The aim of this study was to determine zinc and copper contents in the edible parts (muscle, fillet) of 51 commercially used fish species from the Egypt River Nile (Aswan) and develop analytical method for the determination of zinc and copper by using Differential Pulse Anodic Stripping Voltammetry (DPASV), using an auto sampler[18].

EXPERIMENTAL:

Recommended procedure for determination of copper and zinc in fish samples by differential pulse anodic stripping voltammetry DPASV:

Many studies have already been published on the preparation of samples for wet chemical analysis. Two main techniques have been used: alkali fusion and acidic decomposition or extraction. Alkali fusion induces high blank levels and high detection limits. Due to the small amounts of particulate matter, acidic methods are more suitable. There are many way s to proceed with acids. In addition, various parameters, such as the method of heating, working pressure, and acids, has a role to play in the digestion efficiency. The use of low quantities of acid allows small volumes of final solution to be handled and the detection limits to be improved. The principal acids used for particle digestion are HNO_3, HCl, $HClO_4$, HF, and H_2SO_4. However, the use of HF leads to long, dangerous, and cumbersome schemes and it is not recommended for routine analysis. Thus, in environmental analytical chemistry, acid leaching has become a common procedure as an alternative to total digestion. Five gram of each sample (different weight and length) was weighted in Petri dishes which were put in a closed low temperature (41-45°C) an electrical

furnace for 24 hours and then grinding in an agate mortar. The experimental conditions for the determination of zinc and copper by differential pulse stripping anodic voltammetry are shown in Table (1). The powders of Nile River samples (S_1-S_{17}) and Nasser Lake samples (S_1-S_{12}) were transferred into 100-mL beaker and are mixed with 50 ml (1:1) mixed acid (HNO_3+$HClO_4$) and evaporated to near dryness. The samples were transferred 100-mL measuring flask and diluted to the required volume (100 ml) by bidistilled water. All glassware and polyethylene bottles were soaked in 2M nitric acid for at least 1 week, washed three times with bidistilled water, and finally soaked in 0.1 M hydrochloric acid until being ready for use.

Apparatus:

In this study, DPASV, i.e. differential pulse anodic stripping voltammetry with an EG&G Princeton Applied Research Corp. microprocessor controlled: (PAR) Model 264A stripping analyzer, coupled with a PAR 303A Static Mercury Drop Electrode SMDE, (drop size: medium, area of the drop: 0.014 cm^2). The polarographic cell bottom (PAR Model K 0060) was fitted with an Ag/AgCl saturated KCl, reference electrode, working electrode: HMDE (Hanging Mercury Drop Electrode) and a platinum wire was used as a counter electrode. A PAR 305 stirrer was connected to the 303A SMDE. A PAR Model RE 0089 X-Y recorders was used for the collection of the experimental data for the determination of trace elements. Stirring was performed with a Teflon-coated bar at approximately 400rpm using a magnetic stirrer (KIKA Labortechinik, Germany). A Pyrex glass cell was used for the measurements with magnetic fusion energy (MFE). pH measurements were made with an Orion model

601 digital pH meter. All solutions were prepared with deionised water. Certified atomic absorption spectroscopic standard solutions (1mg/ml) for Cu and Zn were purchased from BDH (UK). Working standard solutions were prepared by appropriate dilution of the stock solutions. A SP1900Pye Unicam Recording Flame Atomic Absorption Spectrophotometer was utilized to measure the concentrations of Cu and Zn Using Pye Unicam Single element hollow cathode lamps.

Samples collected and procedure:

In this work, seventeen fish samples (S_1-S_{17}), belonging to Nile perch fish family from the Nile River in Aswan city (Upper Egypt) and twelve Lates niloticus samples (S_1-S_{12}) from Nasser lake were collected onboard the fishery research vessel, in June 2007. The three parts of each perch fish samples (Head, medium and tail) from South Nile River (Aswan) and Nasser Lake were immediately frozen after suitable preparation onboard and kept in the deep freezer before analyzing. All chemical used were of A.R grade (99.9%) and purchased from BDH, Aldrich, Sigma and Merck.

RESULTS AND DISCUSSION:

Optimization of the solution conditions:

From the previous studies[7], hydrochloric acid is considered as the most suitable supporting electrolyte for the determination of Cu and Zn by DPASV, because it forms chlorocomplexes with these metal ions at low pH values. These chlorocomplexes have different formation constants and hence they will be reduced to their metallic forms at different electrodeposition potentials and then the metals are stripped off the mercury electrode by oxidation. By increasing the

concentration of HCl, separation between the peak potentials (E_p) of Cu and Zn increases gradually up to 0.1M HCl (pH=1) which provides the best peak resolution. This medium is found to be most suitable for the determination of Cu and Zn with good peak separation. The effect of chloride ion concentration on the peak separation was studied as a function of NaCl concentration. It is clear that by increasing the chloride ion concentration, the E_p of Cu shifts to more positive values. The resolution of the peak of Cu is found to increase with increasing the NaCl concentration up to 2M, where the best peak resolution is obtained. This means that the optimal supporting electrolyte for the determination of Cu and Zn by DPASV consists of 0.1M HCl (pH=1) and 2M NaCl. The NaCl added has no effect on the peak resolution of the other elements under investigation.

In this highly acidic medium, a major problem is that the oxidation peak of Zn is masked by the hydrogen evolution. Accordingly, Zn was determined by the same technique (DPASV) after raising the pH of the medium to 4 by adding ammonia/ammonium chloride (as a basic solution).

Optimization of the instrumentation conditions:

The effect of the pulse height on the peak resolution of the investigated elements was studied. Figure (1), shows the effect of pulse height on the separation of Cu, Sb, Bi. It can be seen that, on using pulse height of 25mV, the best peak separation and well-shaped peaks are obtained. Generally, with increasing the pulse height, the peak current of the different elements under consideration increased but the background increased too. So that, 25 mV can be considered as the recommended pulse height

for a good separation and lower detection limit of the two elements under investigation. The effect of scan rate on the resolution of the peak height was studied. The effect of the scan rate on the peak resolution of Cu, Sb and Bi is shown in Figure (2). The most reasonable scan rates for the determination of the two elements are found to be 5–10mV/s, depending on the element determined.

The electrodeposition potentials (E_d) of the two elements were studied separately, and plotted against the peak current (i_p) values to make it easy to select the optimal deposition potential for each metal ion Figure (3). The optimal deposition potentials for metal ions under investigation in the specified solution and instrumental conditions were determined. The relation between the peak current (i_p) and deposition time (t_d) is studied separately for the two metal ions. The linearity is valid for the investigated metal ions through long periods

$$Cu^{2+} + 2e^- + Hg \longrightarrow Cu(Hg) \text{ (preconcentration step)}$$

At the end of the deposition period, the stirring is stopped and after a small rest period (30 s) the potential is scanned anodically to

$$Cu\,(Hg) \longrightarrow Cu^{2+} + 2e^- + Hg \text{ (DPASV step)}$$

2-The pH of the solution is raised up to 4.0 by adding ammonia/ammonium chloride solution (as a basic solution) and then the concentration of Zn can be determined by DPASV after preconcentration at E_d of

$$Zn^{2+} + 2e^- + Hg \longrightarrow Zn(Hg) \text{ (preconcentration step)}$$

$$Zn(Hg) \longrightarrow Zn^{2+} + 2e^- + Hg \text{ (DPASV step)}$$

within the concentration levels normally found in fish samples.

Recommended analysis scheme:

The concentrations of the two elements Cu and Zn dissolved in fish samples can be determined by the method of standard addition under the optimal conditions described above, according to the following analysis scheme:

1-After filtration and acidification of the fish samples to 0.1 M HCl (pH=1), NaCl was added up to 2 M, the investigated solution was deoxygenated for 10 min by purified nitrogen while stirring. Since the metal ions of Cu dissolve in mercury by forming amalgams, they can be determined by DPASV. Preconcentration of this metal is carried out at a potential of –0.067V (vs. Ag/AgCl) at a suitable deposition time, according to the following reaction:
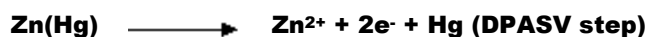
+0.1V with a scan rate of 5mV/s for Cu and a pulse height of 25mV, the dissolved amalgam is then reoxidized back to the following equation:

–1.033 V. After a rest period of 30 s, the voltammogram is then recorded anodically within the potential range –1.30 to –0.70 V with a scan rate of 10mV/s and a pulse height of 25 mV:
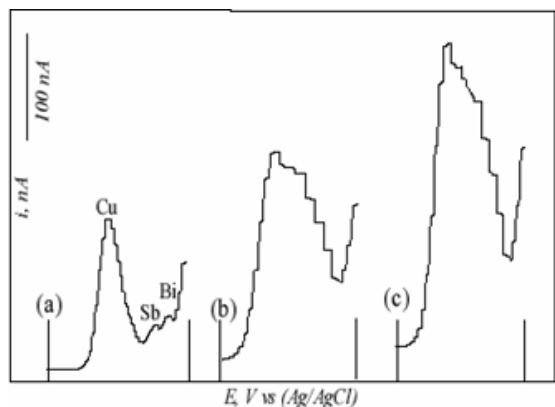
Figure (1): Influence of pulse height on the peak separation of Cu, Sb and Bi in [0.1 M HCl + 2 M NaCl], pH = 1, at E_d = –0.067 V, td =15s and scan rate = 5 mV/s; pulse height: (a) 25 mV, (b) 50 mV, (c) 75 mV 10 mV/s
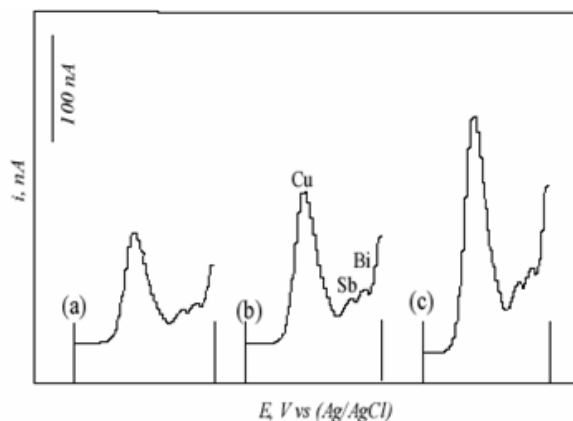
Figure (2): Influence of scan rate on the peak separation of Cu, Sb Bi in [0.1 M HCl +2 M NaCl], pH=1, at Ed = –0.067 V, td=15s and pulse height = 25 mV; scan rate: (a) 2 mV/s, (b) 5 mV/s, (c)
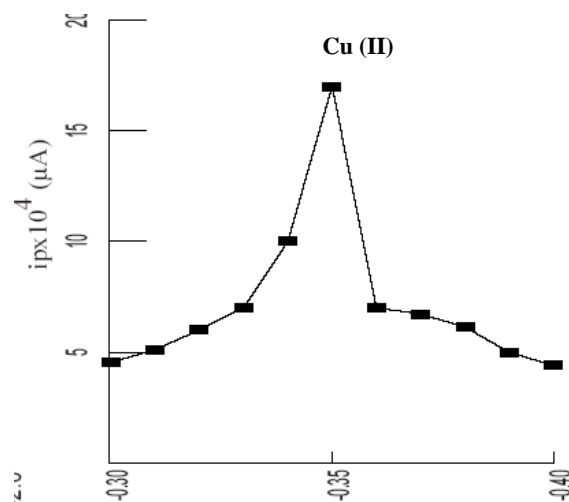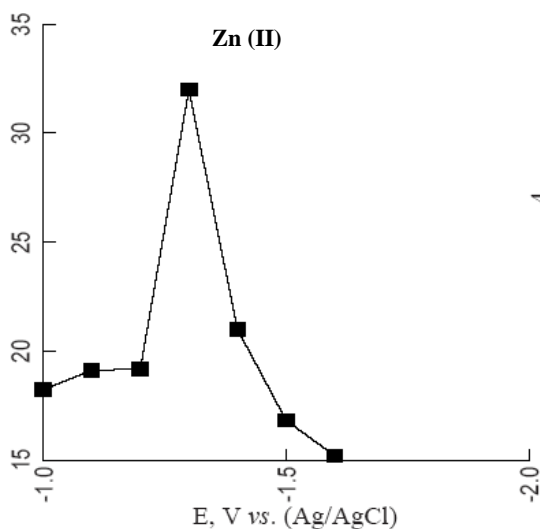

Figure (3): i_p/E_d plot of 0.01mg/L Zn and Cu in [0.1M HCl + 2M NaCl pH = 4 and 1, Scan rate=10 mV/s and t_d = 15s]

Table (1): Experimental conditions for the simultaneous determination of Zn and Cu by differential pulse stripping anodic voltammetry (DPASV)

Condition of DPASV	Zn	Cu
Deposition potential	-1033 mV	-67 mV
Final potential	-1300 mV	-350mV
Deposition time	15 s	15 s
Delay time before potential sweep	10 s	10 s
Potential scan rate	10 mV/s	5 mv/s
Stirring rate	2000 rpm	2000 rpm

Supporting electrolyte: 0.1M HCl   Working electrode: Hanging Mercury Drop Electrode (HMDE)

Test of linearity of calibration plots:

The linearity between i_p and concentration C was tested for the investigated elements by the standard addition method under the optimal conditions Figure (4). Satisfactory linearity was obtained over the concentration range generally found in the fish samples for all investigated elements. The slope values of the calibration curves of the investigated elements are reported in Table (2).

Intermetallic compound formation:

The formation of intermetallic compounds between the investigated metal ions may cause an error in their determination. This aspect was investigated by comparing the slopes of the i_p/concentration plots for each metal present in the solution separately and mixed with 0.1 mg/l of the other investigated metals. Table (2) shows that slope value (a) of the calibration plot of the pure element solution and slope value (b) of the plot of the element mixed with the other investigated element is not significantly different. This indicated that the intermetallic compound formation between the investigated elements in the mercury drop electrode under the optimal conditions of the proposed analysis scheme is small and can be neglected.

Precision, accuracy, detection limits and quantification limit:

Precision and accuracy were determined as relative standard deviation (RSD) and relative error (RE), respectively, by analyzing the same reference standard solution three times for various concentration levels. Table (2) shows RSD and RE for five concentration levels of each element. The detection limits D_L of the investigated metals, defined as the metal concentration yielding an analytical peak equal

to the minimum detectable one, can be calculated as [23]:

$$D_L = 5(S_d/m)$$

Where S_d is the standard deviation of the blank and m is the slope of the calibration line. Table (2) shows the calculated detection limits of all elements under study. The given detection limits of the elements under investigation revealed that the proposed scheme of analysis under the optimal conditions is very sensitive and very useful for ultratrace determination of elements.

The detection and quantification limits have been established as the concentration expressed in nanograms of the element per gram of sample, giving a current reading statistically different from that of the blank, and They have been calculated by dividing 5 and 10 times the standard deviation of the current readings of the blank by the slope of the analytical curve, respectively. From these values, and taking into account the dilution and sample size, the detection and quantification limit in nanograms per gram of sample have been calculated. The values obtained are shown in Table (2). Precision of the method has been estimated from the standard deviation and the correlation coefficient for five replicate analyses of sample solutions, and it provides values greater than 0.006 and 0.99 for all metals, respectively.

Application:

Zinc and copper concentrations of the edible part (muscle, fillet) of Lates niloticus fish belonging to Nile perch family from South River Nile were determined by using DPASV in Figure (5a-b). Zinc concentrations of the South Egypt Nile (Aswan) fishes investigated are given in Table (3). Of all South Nile perch samples, the highest zinc content was found in the muscle of sample 12 in part head (w=320 g, L=29 cm) with 8.745 mg/kg, while in the medium part

samples (2, 10, 13 and 16) the lowest concentration (0.018mg/kg) was detected. It was found, for the samples (S_1 to S_{17}) concentration of Zn (higher in the medium part). The copper concentrations of the South Egypt Nile perch fishes are presented in Table (4). The highest concentration of all samples was found in sample 12 in part head with 0.078 mg/kg and the lowest in sample 15 in part medium, with 0.014 mg/kg. It was also found, for the samples from River Nile, that take (head part) had highest concentration of copper (S_{16} from S_{12}, 0.038 from 0.078mg/kg) and cod had the lowest (S_{14} from S_{15}, 0.032 from 0.014 mg/kg). Correlation coefficients ≈ 0.972 between weight and length. The results showed that the concentrations of, zinc and copper, were higher in sample 12 in part medium fish, than in fish from other sampling locations. The range zinc concentrations for all specimens from South Egypt Nile Perch fishes investigated were 0.018 mg/kg to 8.738 mg/kg and those of copper were 0.014mg/kg to 0.078 mg/kg.

According to the results Tables (3-4), the metal contents in the samples studied depend on the analyzed species. The contents of investigated trace metals in perch samples were found to be in the range 0.0140-0.306 ppm for copper and 0.018–7.178 ppm for zinc. According to these data, zinc has the highest concentration, followed by copper. The highest and lowest copper levels were found in River Nile (Aswan) (Lates niloticus), as 0.306ppm in tail part (FAAS) and 0.0140 ppm in medium part (DPASV), respectively. In the literature, copper levels in perch fish samples have been reported in the range of 0.01–5.33 lg/g. The lowest and highest copper levels in Turkish canned fish samples have been found in the range 7.1–45.7 lg/g[6].

Copper is essential for good health but a very high intake can cause adverse health problems, such as liver and kidney damage[8]. The maximum copper level in permitted for fish is 20 mg/kg according to the Turkish Food Codex[2]. The highest and lowest copper contents were found to be 0.306 (tail part)- 0.014 (medium part) ppm in River Nile (Aswan) Lates niloticus fish and 0068 (head part) -0.017 (medium part) ppm in Nasser lake Lates niloticus fish. In the Figure (6), copper levels in tail perch fish samples have been reported the variance and median 8.01×10^{-5} and 3×10^{-2}, respectively, in Nasser Lake. About zinc levels in River Nile (Aswan) have been reported of the variance and median 4.27×10^{-3} and 0.03, respectively.

Zinc is known to be involved in most metabolic pathways in humans and zinc deficiency can lead to loss of appetite, growth retardation, skin changes and immunological abnormalities. Zinc is widespread among living organisms, due to its biological significance. The maximum zinc level permitted for fish is 50 mg/kg according to both the Turkish Food Codex and MAFF [3-15]. The recommended daily intakes of zinc and copper are 15 mg Zn for adult males and 12 mg Zn for adult females and 1.5–3.0 mg Cu[1].

The lowest and highest zinc contents were found to be 0.018 (medium part)-4.432(tail part) ppm in River Nile (Aswan) Lates niloticus fish and 0.296 (tail part) -7.178 (medium part) ppm in Nasser lake Lates niloticus fish. In the Figure (7), zinc levels in tail perch fish samples have been reported the variance and median 1.787 and 2.048, respectively, in Nasser Lake. About zinc levels in River Nile (Aswan) have been reported of the variance and median 1.6291and 1.258, respectively.
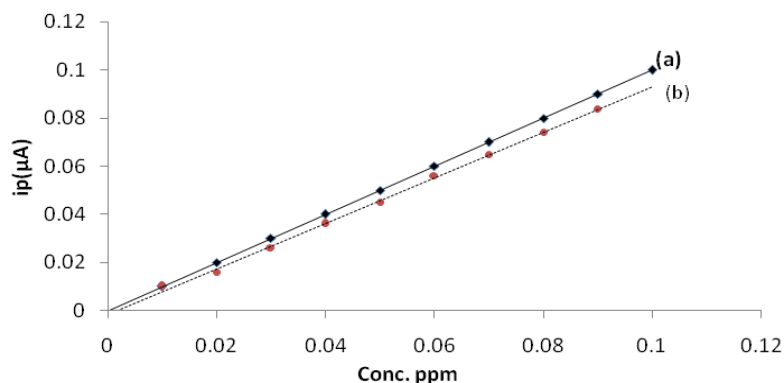
Figure (4): Calibration plot (ip/c) of Cu obtained by standard addition method at Ed = −0.067 V, td = 15 s and pulse height = 25 mV for (a) the element present alone in the solution and (b) the element mixed with 0.1 mg/L of the Zn investigated element
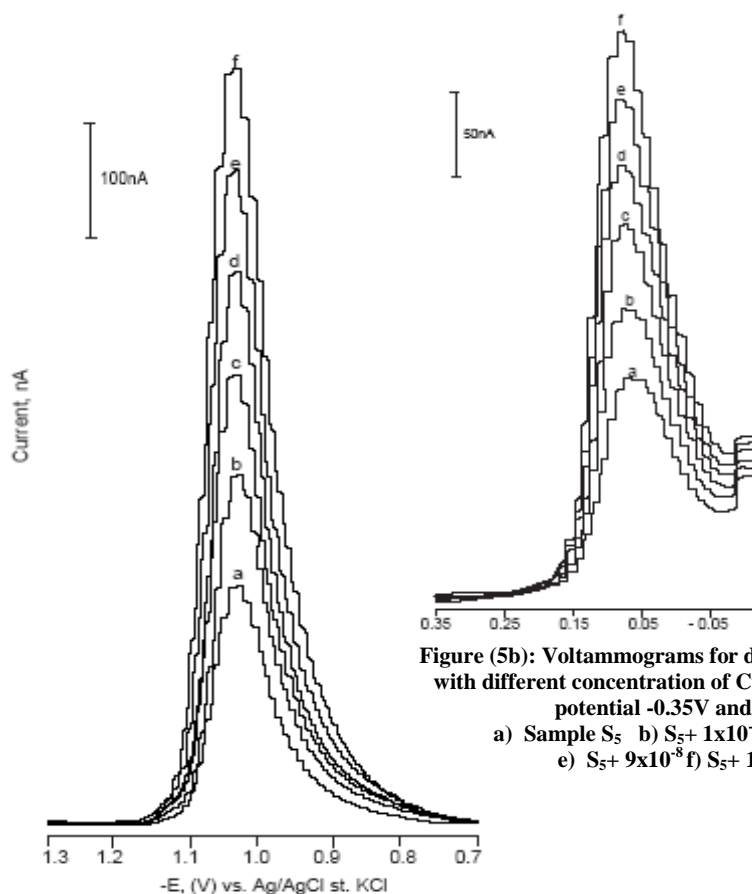

Figure (5b): Voltammograms for determination of Cu(II) in S_5 fish Nile with different concentration of Cu(II) ions 0.1M HCl. pH~1 at initial potential -0.35V and deposition time 15 sec.
a) Sample S_5 b) S_5+ $1x10^{-8}$ c) S_5+ $3x10^{-8}$ d) S_5+ $6x10^{-8}$
e) S_5+ $9x10^{-8}$ f) S_5+ $12x10^{-8}$M of Zn(II) ions

Figure (5a): Voltammograms for determination of Zn(II) in S_5 fish Nile with different concentration of Zn(II) ions 0.1M HCl. pH~4 at initial potential -1.3V and deposition time 15 sec.
a) Sample S_5 b) S_5+ $5x10^{-7}$ c) S_5+ $10x10^{-7}$ d) S_5+ $15x10^{-7}$
e) S_5+ $20x10^{-7}$ f) S_5+ $25x10^{-7}$M of Zn(II) ions

CONCLUSIONS:

In the present study, the samples were lyophilized, milled and then mineralized in a closed low temperature microwave oven. Then the mineralized samples were dissolved in mixed acid (HNO_3 + $HClO_4$) and Zinc and Copper concentrations were determined by DPASV, differential pulse anodic stripping voltammetry by Hanging Mercury drop electrode (HMDE), using an auto sampler. DPASV has some important advantages, such as simultaneous determination of up to 4 elements, low contamination risk and high precision [5]. The contents of investigated trace metals in perch samples (lake Nasser) were found to be in the range 1.09±0.518 (head part)-2.821±1.971 ppm for zinc and 1.048±0.877 (head part) – 1.567±1.392 (medium part) ppm for zinc in River Nile (Aswan). Whereas, the contents of investigated trace metals in perch samples lake Nasser were found to be in the range 0.026±0.006 (medium part)- 0.055±0.006 (head part) ppm for copper and 0.033±0.049 (medium part) –0.049±0.009 (head part) ppm for copper in River Nile (Aswan).

Acknowledgments:

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تقدير الزنك والنحاس في عينات من الأسماك التي تم جمعها من نهر النيل في مصر بواسطة
النبض التفاضلي لفولتامتري النزعي الانودي وامتصاص الطيف الذري

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قسم الكيمياء – كلية العلوم بأسوان – جامعة جنوب الوادي – أسوان – مصر

تحتوي الأجزاء الصالحة للطعام (عضلات, شرائح) المجمعة من ١٧ عينة من أسماك الساموس النيلى في
جنوب مصر (أسوان) على كل من عنصرى الزنك والنحاس، وقد تم تقديرها بواسطة طريقة النبض التفاضلي
لفولتامتري النزعي الانودي (DPSAV). وقد اتبع في خطوة تحضير العينات أن يتم تجميع العينات المجففة من
الأسماك أولا, ثم تطحن في مطحنة، وأخيراً يتم هضمها عن طريق استخدام مخلوط من حامضي النيتريك
والبركلوريك المركزين (١:١). وتم تقدير دقة التركيزات في هذه الدراسة بواسطة فحص القياسات المعتمدة من
المواد المرجعية (رقم ٤٢٢، العضلات) من لجنة الاتحاد الأوروبى. كما لوحظ أن جميع تركيزات الزنك والنحاس
التي تم تقديرها في هذه الأنواع من الأسماك في نهر النيل في مصر أنها مصدر جيد لهذه العناصر الأساسية في
تلك المنطقة، وأن هذه الطريقة المطورة مناسبة للتحليل الروتيني لهذه العينات. ومن المميزات الكبرى لطريقة
التحليل (DPSAV) هي البساطة، والانتقائية، والحساسية، واختصار وقت التحليل باستخدام جهاز مطياف
الامتصاص الطيف الذري (AAS).