
Levels of Heavy Metals in Blood of Electronic Technicians in Port Harcourt Metropolis, Rivers State, Nigeria

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ABSTRACT: *In human, blood is a body fluid that circulate over the body and transport nutrients and other substances like oxygen, hormones, waste products among others to the cells and organs. It is a dynamic component for the survival and functioning of living organisms. Thus, the levels of heavy metal in the blood of Electronic Technicians were investigated during the dry season of 2021. Forty (40) blood samples from Electronic Technicians were purposively collected with the aid of 5ml needle and syringes into EDTA bottles and placed in an ice-chest, and were taken into laboratory for further analysis of Lead (Pb), Mercury (Hg) and Arsenic (As) respectively. Non-experimental method like interview and observational technique (number of years, age and marital status) were also utilized. The Flame Atomic Absorption Spectrophotometry (AAS), Descriptives Statistics, Single Factor analysis of variance (ANOVA) and SPSS version 23 were used for the organization of data. Result inter-alia reveals that the mean lead (Pb) of 0.64218 ± 0.9245 (mg/L) is high. The working experience range of exposure for lead (Pb) revealed further that 11-20years has the highest bioaccumulation value of 12.41mg/L followed by 1-10years having 6.65mg/L respectively. The highest mercury value in the blood of Electronic Technician in this study was 4.45mg/L for an exposure-years of 1-10years working experience while the least mercury value in the blood of Electronic Technician in this study was 0.10mg/L for an exposure-years of 41-50years working experience. The value of arsenic in the blood of Electronic Technicians from 11-12years of experience had the highest value of bioaccumulation of 8.47mg/L followed by 1-10years which recorded 3.02mg/L. These values of lead, mercury and arsenic as observed in this study serves as risks factors to the recipient's health status since they are of higher values to the reference factor. Thus, electronic technician should undergo intensive medical attention to ascertain their health status with heavy metal. Both government and nongovernmental agencies should create sensitization and awareness campaign on the need for the utilization of personal protective devices at work environment no matter the nature of the small and medium-scale enterprises.*

KEYWORDS: Electronic Technician Blood, Serum, Heavy metal, Bio-accumulation, bio-availability, Exposure rate

INTRODUCTION

Heavy metals are naturally occurring elements that have a high atomic weight and a density at least 5 times greater than that of water [1, 2]. Albeit, their multiple utilization in industrial, domestic, agricultural, medical and technological applications have led to their wide distribution in the environment. In modern day society, there is a rising concern over their potential effects on human health and the environment [3] including their toxicity which depends on several factors such as dose, route of exposure, and chemical components, as well as the age, gender, genetics, and nutritional status of exposed individuals [1].

According to [4] human beings are exposed to a high level of toxic and heavy metals resulting from many sources, which may include the burning of coal, through natural gas and petroleum exploitation, including incineration of waste materials worldwide. Such metals may have contributed negative influences on aging, diseases, and even genetic defects. Thus, accurate and precise determination of these elements in the human fluids and tissues becomes inevitable. However, in the field of forensic and toxicological clinical chemistry, the determination of the toxic heavy metals, minor minerals, and even major elements in the human body is critical [5]. In public and environmental health, the high degree of toxicity of arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb), and Mercury (Hg) are among the most priority metals. These metallic elements are considered systemic toxicants that are known to induce multiple organ damage, even at lower levels of exposure [1, 6, 7, & 8]. The minor minerals like manganese (Mn), iron (Fe), cobalt (Co), copper (Cu), and zinc (Zn) which are present in lower levels are also essential as metabolic agents as well as enzymes catalysts. Heavy metals have no function in the body and can be highly toxic. They occur near the bottom of the periodic table, and are non degradable [1, 9]. Based on their extreme toxicity, toxic and heavy metals must be detected at very low levels in the human fluids and tissues. Nonetheless, blood and hair are the most suitable human biological samples generally used in such metal analysis. More so, due to the partitioning of the toxic and heavy metals in their hair, recently hair samples have been used as good index in such studies [4].

Heavy metals are highly dangerous even in trace levels and could cause chronic acute poisoning. These metals are harmful and have no known function in the human body. The toxicity of these metals can result in many illnesses such as reduced or damaged mental and central nervous function, lower energy levels, and damage to blood composition, lungs, kidneys, liver, and other vital organs. Literature holds that long-term exposure to such elements (heavy metal) may result in slowly progressing physical, muscular, and neurological degenerative processes that mimic Alzheimer's disease, Parkinson's disease, muscular dystrophy, and multiple sclerosis [5, 10, 11 & 12]). Among these heavy metals, cadmium and lead are carcinogenic and the most toxic metals, and the cardiovascular effects of such two metals in humans come from their association with increased blood pressure [5].

Heavy metals enter the human body through many sources which are not limited to houses paint, fish, dental amalgam, farming, mining and smoking including second hand smoke. Smoking is an important source of exposure to nickel, cadmium, lead, and other toxic metals. Once inhaled through smoking, toxic metals have a long biological life span. Chronic adverse effects on human health may, therefore, in later years result from prolonged intake of such toxic elements, some of which are powerful carcinogens [5, 13]. At very high levels, most heavy metals can cause health problems [9]. Again, in Port Harcourt, the small and medium-scale enterprise (SMEs) abound, and the electronics technician are part of these group. Most of these technicians are not literate and thus lack occupational safety and management in relation to the exposure to these heavy metals. They operate without such consciousness and may have inadvertently injected these heavy metals by an act of omission or commission towards safety. This therefore underscores the nexus upon which the study is been guided. Thus, to ensure efficiency and effectiveness of the study both experimental and non-experimental research method is adopted in this study.

METHODOLOGY

Study Area

The geographical coordinate of Port Harcourt lies between latitude $4^{\circ}49'27.0012''$ N and longitude $7^{\circ}2'0.9996''$ E respectively, and lies 9 meters above sea level. It has a tropical climate with significant rainfall pattern in most months of the year. The average annual temperature is 26.4°C or 79.5°F with precipitation of about 2708 mm or 106.6 inches per year. Most precipitation occurs in September with an average of 141 mm or 16.3 inches. Driest month is January with 36 mm or 1.4inch rainfall. Again, the warmest month of the year occurs in February with an average temperature of 26.70°C or 81.7°F while August serves as the coldest month, with an average temperature of 25.2°C or 77.4°F . Temperature varies by 2.4°C or 36.3°F throughout the year [14].

Sample Size and Sampling Method

A purposive sampling method was used to select the area. A sample size of 40 volunteers were gotten from the total population of 300. Convenience method was used to select technicians who accepted and allowed their blood samples to be collected.

Collection of Sample

A 5mls needle and syringe each was used to collect venous (blood through venous puncture) from the volunteered electronic technicians to be sampled. Each blood samples collected were subjected into EDTA bottles with the use of a 5ml syringe, and were transferred into ice packed cooler to the laboratory.



Figure 3.1: Map of Port Harcourt Metropolis

Laboratory Apparatus

250ml Analysis Beaker (Digestion vessel hot plate or heating mantle Pipette), 5ml and 10ml Pasture pipettes, graduated measuring cylinder (50ml/ 100ml) - Graduated once Funnel, Beakers (50ml, 100ml), Watchman filter paper (0.2mm), Wash bottles.

The Reagents:

Distilled Other reagents include: distilled water from ASTM (Graded quality), Concentrated Perchloric acid (Analytical grade) and Concentrated Nitric Acid (HNO_3) (Analytical grad)

Laboratory Procedure

The ice chest blood samples were allowed to acclimatize to ambient temperature. 1-2ml blood samples were measured into the digestion vessels. 6ml of concentrated Nitric Acid was added with 2ml of concentrated Perchloric Acid, and the solution were mix with 20ml of distilled water. It was allowed to settle half an hour before taken to the hot plate for the digestion to complete (i.e., the pale-yellow coloured solution was reduced to 1/3 of its original volume), and was later allowed to cool for some minutes and were transferred and filtered via watchman filter paper of 0.2mm that had been inserted into the funnel. Distilled water was use to rinse the digestion vessel into the measuring cylinder through the filter paper in the funnel with the aid of wash bottle. Aliquot for aspiration with Flame Atomic Absorption Spectrometer (AAS) in the 50ml plastic bottle with cellophane cap were observed while utilizing ASTM 2011 Edition. More so, the Solar Thermos-Elemental AAS Model SE-7I906 was used in determining the content of heavy metals in the Acid

digested samples.

Data Analysis

The statistical analysis was done with the use of Microsoft Excel 2007 and Statistical Package for Social Scientists (SPSS) version 23. The statistical technique used includes: Descriptive statistics, coefficient of variance and standard error to validate the correctness of the work, single factor ANOVA using F distribution was used to determine if there is significant difference in the level of exposure to the different metals. The individual students t-test was used to determine significant difference in the mean concentration of these metals. The ANOVA was also used to determine the post hoc Multiple tests for Heavy Metals in the exposed groups.

Ethical Clearance/Informed Consent

The protocol and clearance for this study was received from the Rivers State Ministry of Health, and the individuals were met personally to inform them on the need and importance of the research. An Oral informed consent was obtained from each subject used for this study, after deliberations with the members to be used for this work.

RESULT

Nuptial Status of Electronics Technicians

Table 1 revealed that 28 of the respondents under study making about 70% of the total population are married, and about 12 of the respondents making about 30% of the total population are not married. Furthermore, the married population is greater than the unmarried population, meaning that more responsible people were sampled. The unmarried people could be apprentices or member of the family business who have also come to learn the trade.

Table 1: Marital Status of Electronics Technicians

| Status | Number | Percentage |
|--------------|-----------|-------------|
| Married | 28 | 70% |
| Single | 12 | 30% |
| Total | 40 | 100% |

Source: Researcher's Field Survey, 2022

Sex Status of Electronics Technicians

The result revealed that all the respondents were male 40 (100%) without any female in the study group. This implies that the work is predominantly masculine (Table 2).

Table 2: Sex of Electronics Technicians

| Sex | Number | Percentage |
|--------|--------|------------|
| Male | 40 | 100% |
| Female | NIL | NIL |
| Total | 40 | 40 |

Source: Researcher's Field Survey, 2022

Age Distribution of Electronics Technicians

The result shows that there are no technicians with ages bracket 1 – 20 years. However, those with ages bracket 21 – 40 years have 17 (42.5%) of the total number of respondents while those with ages bracket 42 to 60 also had 17 (42.5%). More so, those with ages 61 – 80 years had 6 (15%) and of the total population under study. This implies that 34 out of 40 technicians with ages between 21 and 60 dominated the work compared to 6 from ages 61 to 80 (Table 3).

Table 3: Respondents of Electronics Technicians by Age

| Age Range | Frequency (f) | % Distribution |
|-----------|---------------|----------------|
| 1 – 20 | - | - |
| 21 – 40 | 17 | 42.5 |
| 41 – 60 | 17 | 42.5 |
| 61 – 80 | 6 | 15 |
| 81 – 100 | - | - |
| | N = 40 | N = 40 |

Source: Researcher's Field Survey, 2022

Bio-accumulation of Heavy Metals in the different Groups

The bio-accumulation levels of the heavy metals in blood shows variations among the electronic technicians based on their year(s) of work (electronic repairs and maintenance). Furthermore, 1-10years and 11-20years had 10(25%) frequency each while 21-30 had whereas 41-50 years and 51-60years had frequency of 2(5) and 4(10) respectively. However, work experience between 31-40years had no frequency of responses (Table 4). In same manner, the degree of accumulation varied across metals in spite of the working experiences. Lead, Mercury and Arsenic had 6.65mg/L, 4.45mg/L and 3.02mg/L correspondingly for working age experience between 1-10years whereas for the age experience between 11-20years Lead had 12.41mg/L, Mercury had 0.34mg/L and Arsenic had 8.47mg/L respectively. More so, 3.17mg/L, 0.37mg/L and 1.60mg/L corresponded with lead, Mercury and Arsenic for work experience age between 21-30years (Table 4). Finally, 0.12mg/L, 0.10mg/L and 0.53mg/L represents Lead, Mercury and Arsenic for years of experience of 41-53years while 0.24mg/L, 0.21mg/L and 0.53mg/L represent Lead, Mercury and Arsenic present in the blood of electronic workers with age experience between 51-60years harmoniously (Table 4).

Table 4: Bio-accumulation of Heavy Metals in the different Groups

| Range for years of working experience | Frequency (f) | Percentage (%) | Amount accumulated | | |
|---------------------------------------|---------------|----------------|--------------------|------|------|
| | | | Pb | Hg | As |
| 1 – 10 | 10 | 25 | 6.65 | 4.45 | 3.02 |
| 11 – 20 | 10 | 25 | 12.41 | 0.34 | 8.47 |
| 21 – 30 | 14 | 35 | 3.17 | 0.37 | 1.60 |
| 31 – 40 | NIL | NIL | - | - | - |
| 41 – 50 | 2 | 5 | 0.12 | 0.10 | 0.27 |
| 51 – 60 | 4 | 10 | 0.24 | 0.21 | 0.53 |
| | N = 40 | N = 100 | | | |

Source: Researcher's Field Survey, 2022

Correlation between the years of Exposure and Lead (Pb)

The multiple correlation between the years of exposure of Pb is 0.461 while the coefficient of determination is 0.212 which is about 21.2% of the variation of Pb in the blood of electronic workers. This can be explained by the number of years of exposure. The adjusted R-Square is 0.1913 with a standard error of 13.518. The result revealed that there was no correlation between the years of exposure with bioaccumulation of these metals since the coefficient is less than 5 (Table 5).

Table 5: Summary output for Exposure to Lead (Pb)

| | |
|------------------------------|-------------|
| Regression Statistics | |
| Multiple R | 0.4605331 |
| R Square | 0.212090736 |
| Adjusted R Square | 0.191356282 |
| Standard Error | 13.51861539 |
| Observations | 40 |

Source: Researcher's Field Survey, 2022

Correlation between the years of Exposure and Mercury (Hg)

The result depicted that the multiple correlation between number of years exposed to the amount of Hg is 0.2623, and the coefficient of determination is 0.688 (6.88%) of the variation of Hg in the blood for the number of years exposed. The adjusted R-Square is 0.0443 with a standard error of

14.696 (Table 6). Furthermore, there was no correlation between the years of exposure with bioaccumulation of these metals since the coefficient is less than 5 (Table 6).

Table 6: Summary output for Exposure to Mercury (Hg)

| Regression Statistics | |
|------------------------------|-------------|
| Multiple R | 0.262330884 |
| R Square | 0.068817493 |
| Adjusted R Square | 0.04431269 |
| Standard Error | 14.69641859 |
| Observations | 40 |

Correlation between the years of Exposure and Arsenic (As)

The correlation between number of years for exposure levels to As is 0.2673 while the coefficient for determination is 0.0715 (7.15%) for the variation of As in the blood of electronic technicians for the number of years exposed. The adjusted R-Square is 0.0470 with a standard error of about 14.675 which defined no correlation between the years of exposure with bioaccumulation of these metals since the coefficient is less than 5 (Table 7).

Table 7: Summary Output for exposure to As

| Regression Statistics | |
|------------------------------|-------------|
| Multiple R | 0.267356984 |
| R Square | 0.071479757 |
| Adjusted R Square | 0.047045014 |
| Standard Error | 14.67539492 |
| Observations | 40 |

Source: Researcher's Field Survey, 2022

Variation of Heavy Metal Levels in Serum of Electronic Technician

The mean concentration of Pb was 0.64218 ± 0.09245 ; Hg had 0.134505 ± 0.05766 and As had 0.399275 ± 0.107670 congruently (Table 8)., The result furthermore revealed that, there was statistically significant difference in the mean concentration of these metals [$F_{(2, 117)} = 8.243$, $P = 0.000$] in the blood of electronic technicians at $P < 0.005$ (Table 8).

Table 8: ANOVA for Metal Levels in Electronic Technicians

| Metals | | Mean ± SEM | |
|----------------|----------|-------------|---|
| Pb | | 0.64218 | ± |
| | 0.09245 | | |
| Hg | | 0.134505 | ± |
| | 0.05766 | | |
| As | | 0.399275 | ± |
| | 0.107670 | | |
| F | | 8.243 | |
| P-Value | | 0.000 | |
| Remain | | Significant | |

Significant (at $P < 0.005$)

DISCUSSION

Blood is a body fluid in humans that circulate over the body and transport or distribute nutrients and other substances like oxygen, hormones, waste products etc., to the cells and organs [15, 16]. It is a vital component for the survival and functioning of living organisms [17]. The amount of heavy metal in the blood of Electronic Technician could be influenced by the degree or rate of exposure to these heavy metals either by inhalation, ingestion or injection by the recipient. However, heavy metal has been proven to be a major threat to human health due to their ability to cause membrane and Deoxyribonucleic acid (DNA) – a molecule that carries genetic information for the development and functioning of an organ damage, and to perturb protein function and enzyme activity.

Electronic technicians are one of the groups of medium and small-scale enterprise (SMEs) that deals with repairs of faulty electronics, spanning from television (TV), radio, electric fans, home theater, blue-tooth, Air condition (AC) and other related appliances. Most of these group of skill professionals lacks occupational safety and personal hygiene since they do not go through formal education. Thus, they may have been inadvertently exposed to some of these heavy metals since most of the electronics components are made up of copper (Cu), lead (Pb), zinc (Zn), aluminum (Al), mercury (Hg), arsenic (as) and so many others. The degree of exposure could slightly be related to the amount of heavy metal present per unit area per unit time. This could enhance and promote bioaccumulation, bioavailability and bio-magnification if all other variables factors remain constant in the recipient serum.

Furthermore, in the current study, out of forty respondents' twenty-eight 28(70%) were married and 12(30%) were unmarried. Again, the result indicated that all the respondents were male 40(100%). This simply reveals that Electronic Technician is a male dominated profession in this part of the country. The high percentage of married respondents could serve as a precursor that any negative impact due to heavy metal on the recipient could accentuate the economy life wire of the home of the Electronic Technician as most women are not independent.

Lead (Pb) is a toxic metal that is harmful to human health even at very low level. Authorities have averred that lead (Pb) is one of the persistent metals that can bioaccumulate in the body of human over time [18, 19, 20, 36]. In the current study, the mean lead (Pb) of 0.64218 ± 0.9245 (mg/L) was recorded. Again, the working experience range of exposure for lead (Pb) revealed that 11-20years has the highest bioaccumulation value of 12.41mg/L followed by 1-10years having 6.65mg/L respectively whereas the least level of 0.12mg/L was recorded for 41-50years. These values are far higher when compared with the blood lead reference value (BLRV) for children $<3.5\mu\text{g/dL}$ [21, 22, 23] and thus could trigger or influence the affected individual's health status negatively.

Furthermore, mercury (Hg) is a chemical element (the only common metal that is liquid at ordinary temperature), commonly known as quicksilver, heavy, and silvery-white liquid metal. However, mercury can cycle in the environment as part of natural and anthropogenic activities. Mercury metal is highly useful in electrical devices and electrochemistry. It is a volatile liquid and has a measurable vapour pressure at room temperature. It has a density of 13.6 g.cm^{-3} at 20°C . Small amounts of mercury are often present in blood, basically due to variable amounts of methylmercury in the diet. Typical levels of total mercury in blood range up to about $5\mu\text{g/L}$. The mercury concentration in whole blood is usually lower than $10\mu\text{g/L}$, but the value of $20\mu\text{g/L}$ or below is considered normal [24]. However, The International Commission on Occupational Health (ICOH) and the International Union of Pure and Applied Chemistry (IUPAC) set the average blood mercury concentration level in those who do not eat fish at $2\mu\text{g/L}$. Such blood and urine mercury concentration levels signify background concentrations, the mean concentration level in the general population [24, 25, 26]. Blood mercury concentration levels maintained a high level even when the exposure has ceased, due to the heavy burden of mercury on the body for those chronically exposed to mercury [25, 26]. The highest mercury value in the blood of Electronic Technician in this study was 4.45mg/L for an exposure-years of 1-10years working experience. Elsewhere in Korea, values ranged from $4.28\mu\text{g/L}$ in 2009 and $3.64\mu\text{g/L}$ in 2010 respectively. In United State blood mercury concentration was $0.86\mu\text{g/L}$, Germany ($0.58 \mu\text{g/L}$) and Canada $0.76\mu\text{g/L}$ respectively [22, 27, 28, 29, 30 & 31]. These values contrast the values obtain in the current study. The high values obtain in this study could be due to deficiencies in the application of personal protective devices by the recipient (Electrician Technician). This could have a debilitating health consequence on the exposed Electrical Technician possibly in the nearer future if no health assistance in terms of mitigation and treatment is observed.

Arsenic is one of the heavy metals that affects humans when been exposed to. The arsenic cycle has been enlarged as a consequence due to human interference and thus more amount end up in the environment and living organisms. Usually, it is emitted by copper (Cu) producing industries, including Pb and Zn production. It cannot be destroyed once it has entered the environment hence the amount we add can spread and cause health effects to human and animals according to [32]. In the blood of human, soluble inorganic arsenic can cause immediate toxic effects. Ingestion of large amount can lead to gastrointestinal symptoms like severe vomiting, disturbances of blood and circulation, damage to nervous systems and possibly death [32]. Blood arsenic levels in healthy subjects vary substantially with exposure to arsenic in the diet and the environment [33]. Blood

arsenic is for the detection of recent exposure poisoning only. According to [34], the potentially toxic range for blood arsenic is greater or equal to 600µg/L. The arsenic load in Electrical Technician in these current holds that 8.47mg/L of arsenic was recorded as the highest concentration load for work experience range of 11-20years while the least value of 0.27mg/L was recorded for 41-50years of work experience. Considering [34] as a reference point, the work experience by age ranges from 1-10years, 11-20years and 21-30years are at risks. Organic arsenic can cause neither cancer, nor DNA damage [35]. The observed high values of arsenic recorded in this study could be due to lack of educational awareness and poor sensitization on one hand and ignorance on occupational safety by the recipient on the other hand.

SUMMARY OF FINDINGS

1. The mean lead (Pb) of 0.64218 ±0.9245 (mg/L) in this study was high.
2. The working experience range of exposure for lead (Pb) revealed that 11-20years has the highest bioaccumulation value of 12.41mg/L followed by 1-10years having 6.65mg/L respectively.
3. The least level of 0.12mg/L of Pb was recorded for 41-50years working experience.
4. All the values recorded in this study are far higher when compared with the blood lead reference value (BLRV) for children <3.5µg/dL (NHANES, 2018; CDC, 2012; LEPAC, 2021).
5. The highest mercury value in the blood of Electronic Technician in this study was 4.45mg/L for an exposure-years of 1-10years working experience.
6. The least mercury value in the blood of Electronic Technician in this study was 0.10mg/L for an exposure-years of 41-50years working experience.
7. These values of mercury obtain in the current study contrast the values obtain Korea4. (8µg/L), US (0.86µg/L), Germany (0.58µg/L) and Canada (0.76µg/L) respectively.
8. The high values of mercury obtain in this study could be due to deficiencies in the application of personal protective devices by the recipient (Electrician Technician).
9. The high value of mercury could have a debilitating health consequence on the exposed Electrical Technician possibly in the nearer future if no health assistance in terms of mitigation and treatment is observed.
10. The value of arsenic in the blood of Electronic Technicians from 11-12years of experience had the highest value of bioaccumulation of 8.47mg/L followed by 1-10years which recorded 3.02mg/L.
11. These two values of arsenic as recorded in this study are risks factors to the recipient's health status since they are of higher values to the reference factor.

CONCLUSION

Electronic technicians are group of medium and small-scale enterprise (SMEs) that deals with repairs of faulty electronics, spanning from various electronic appliances which usage is either domestic or industrial or both. Mercury is a liquid metallic element with many useful applications. It is also an element with hazardous properties to the environment and workplace, including

corrosiveness to so many materials. Arsenic (As), lead (Pb) and mercury (Hg) are within the 10 chemicals of major public health concern (WHO, 2021). Notwithstanding the fact that the toxicity of these elements is well-known, their various technological, medical and agricultural, applications cause still a huge threat to human health. Lead and Arsenic are ricks factors to human health (digestive, urinary, DNA Carcinogenic among others). However, most of these group of skill professionals lacks occupational health and safety, environmental sanitation and personal hygiene knowledge since they do not go through formal education. Considering the adverse consequences of these heavy metals, it is therefore hoped that Electronic Technicians should be sensitized on occupational health and safety as it related to their profession.

RECOMMENDATION

1. Electronic Technicians should be encouraged to undergo periodic medical check-up.
2. Government should encourage training SMEs with short occupational safety courses.
3. Electronic Technician should be encouraged to the utilization of personal protective devises while on duty.
4. There should be continuous education and sensitization awareness campaign on the promotion of occupational health and safety by governmental and non-governmental agencies at all levels of the electronic technician chain of productivity of the SMEs.

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COMPETING INTEREST

Authors have declared that no competing interests exist.

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